Supporting Information

Tuning of Hydrophobic-Hydrophilic Balance for the Development of a Salt-Tolerant and Protease-Resistant Lipopeptide AMP

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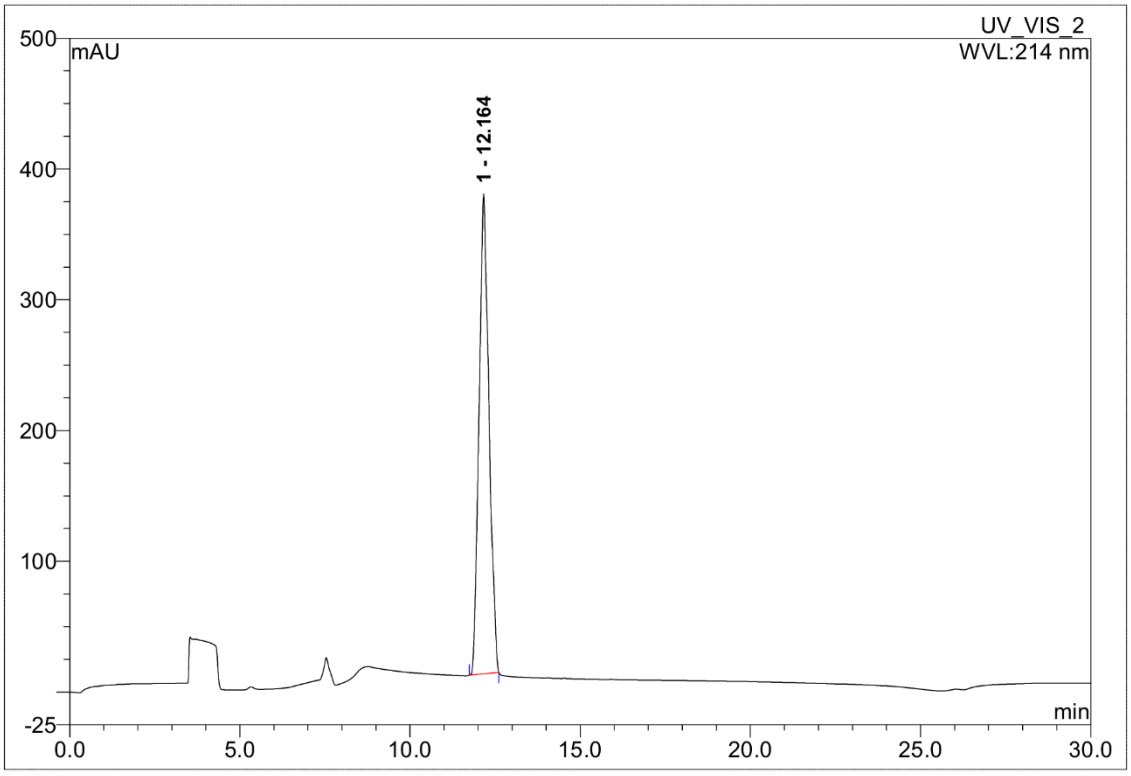

Figure S1. Analytical HPLC trace for **P8**.

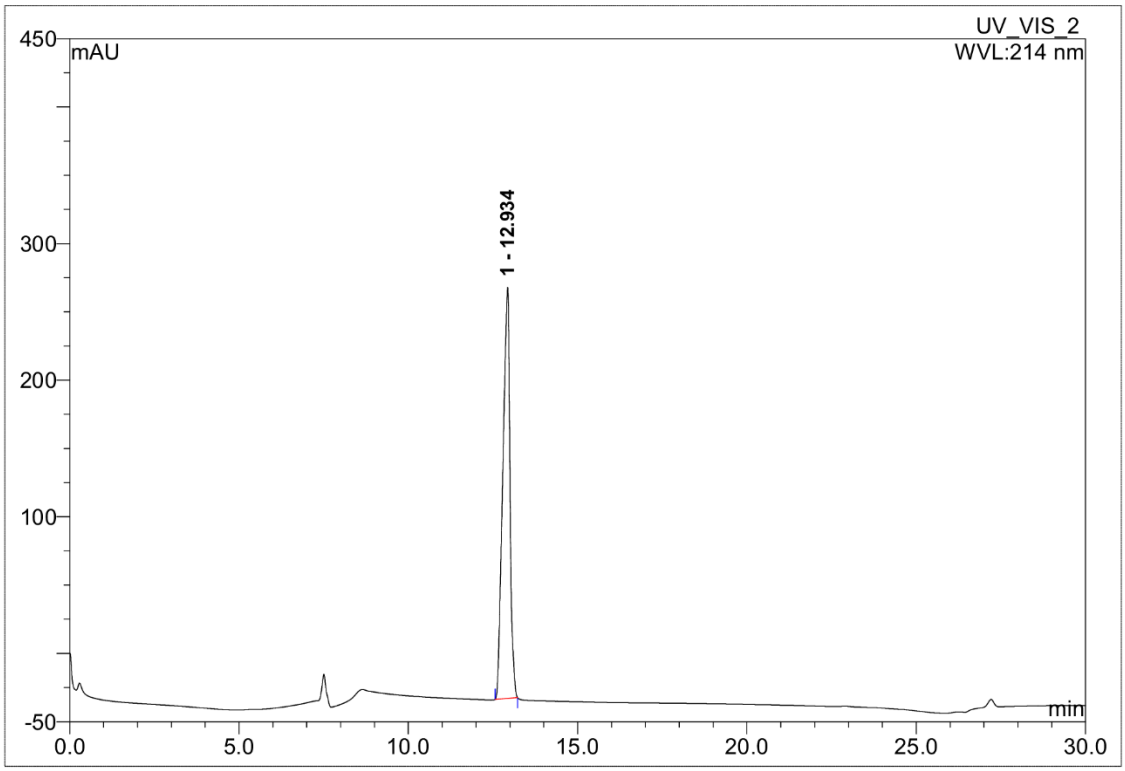

Figure S2. Analytical HPLC trace for **P10**.

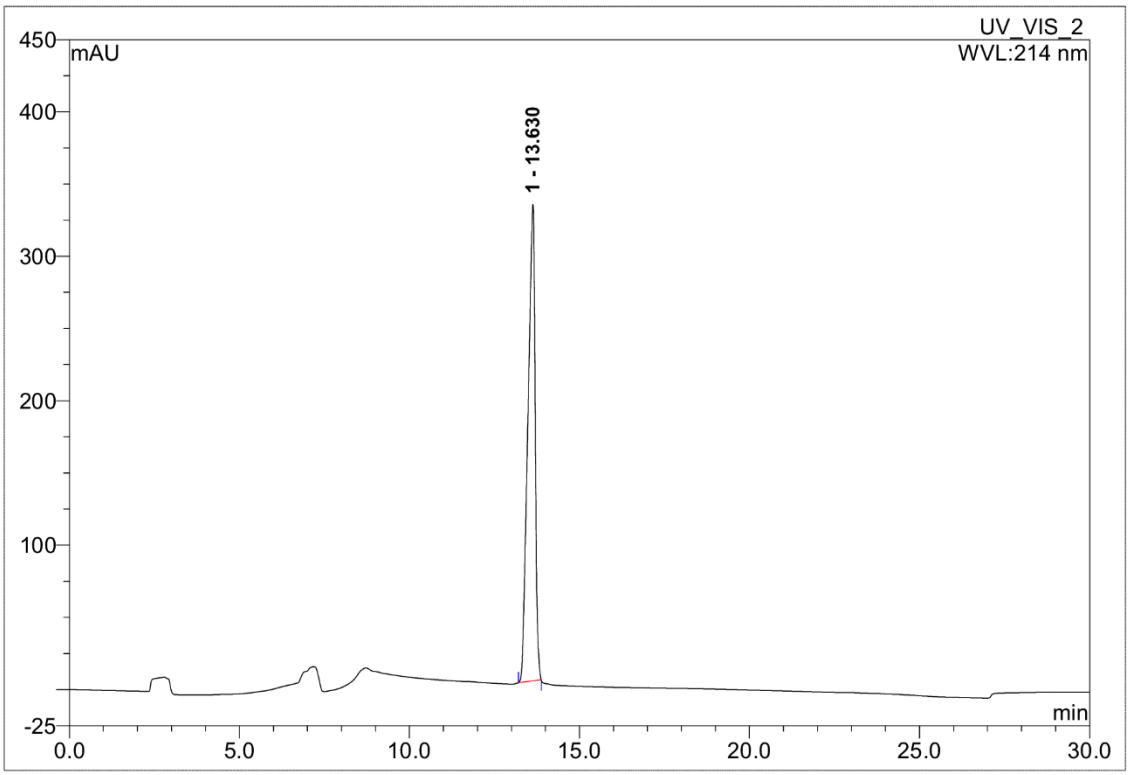

Figure S3. Analytical HPLC trace for **P12**.

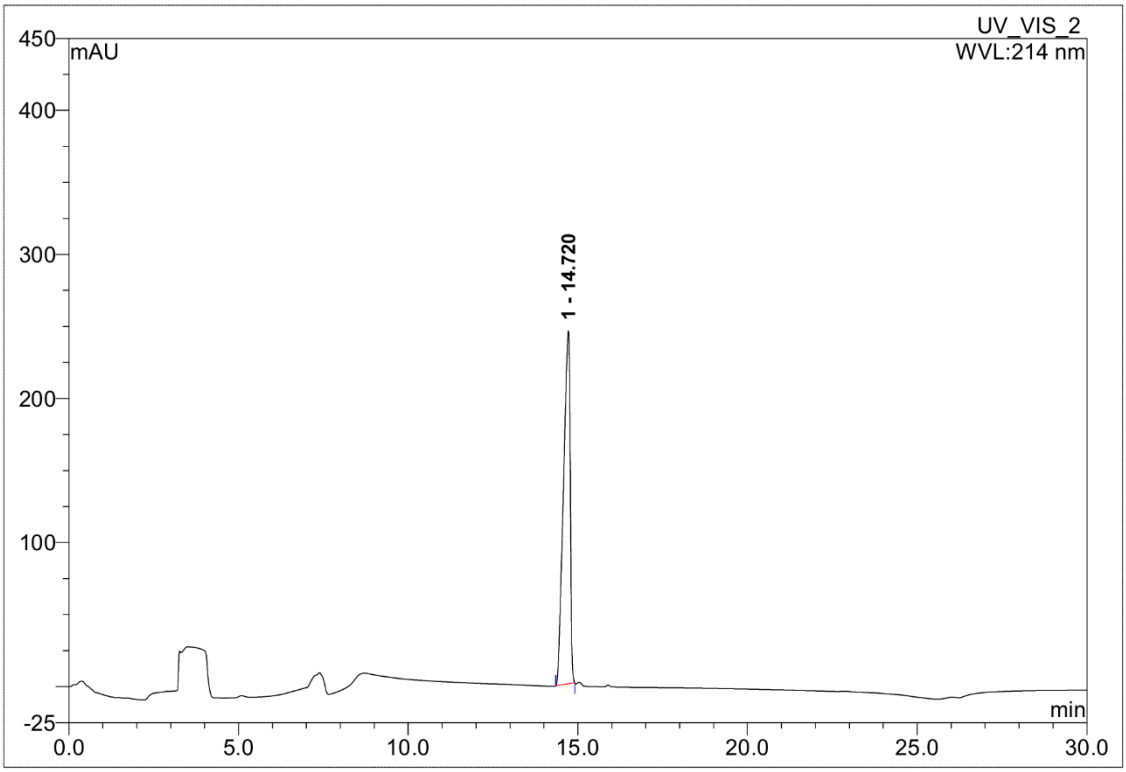

Figure S4. Analytical HPLC trace for **P14.**

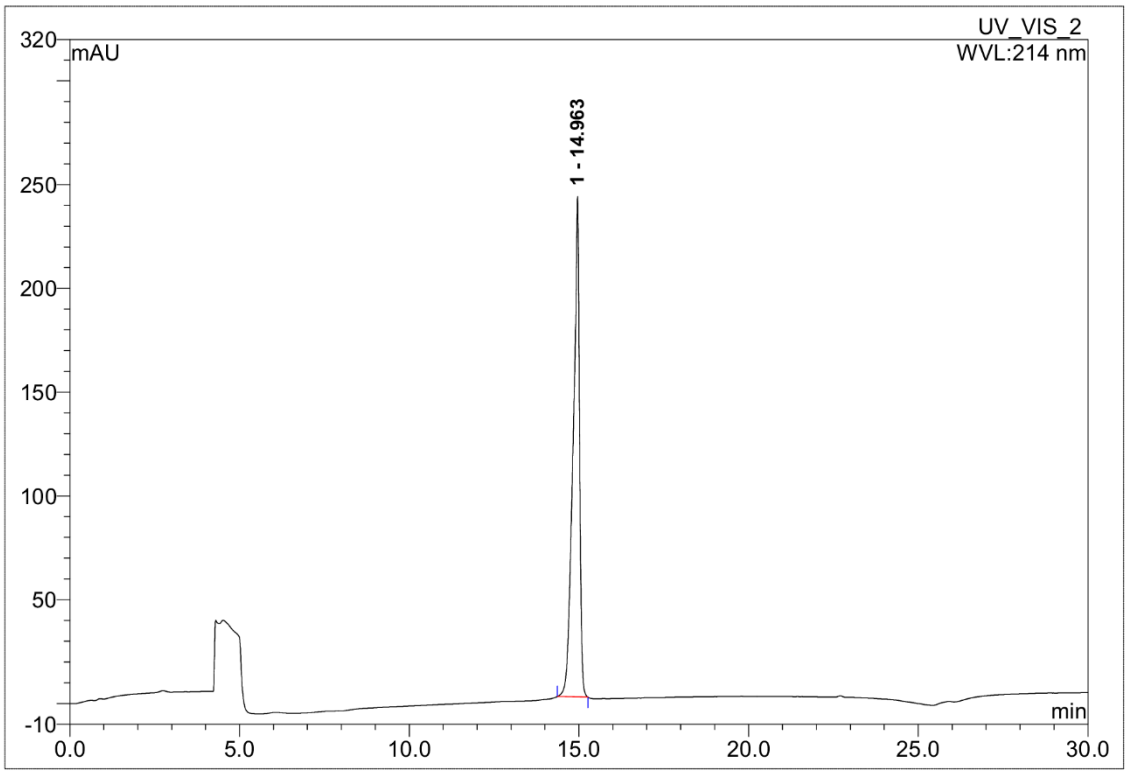

Figure S5. Analytical HPLC trace for **P16.**

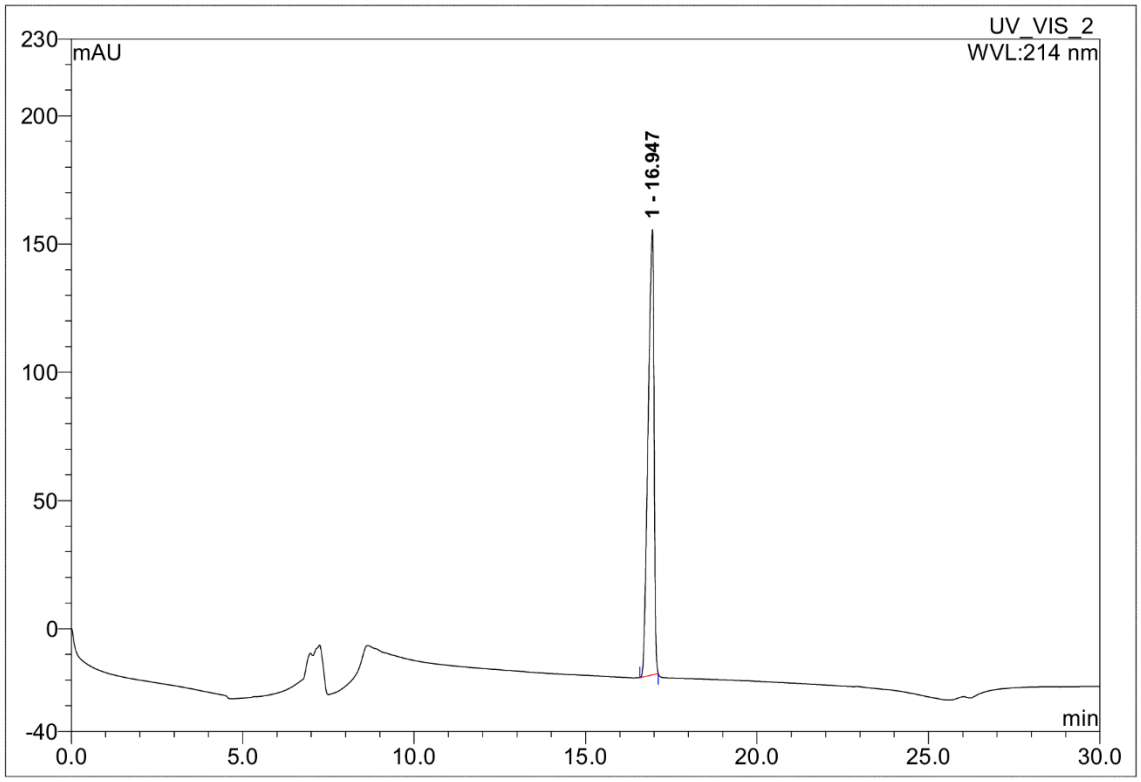

Figure S6. Analytical HPLC trace for **P18**.

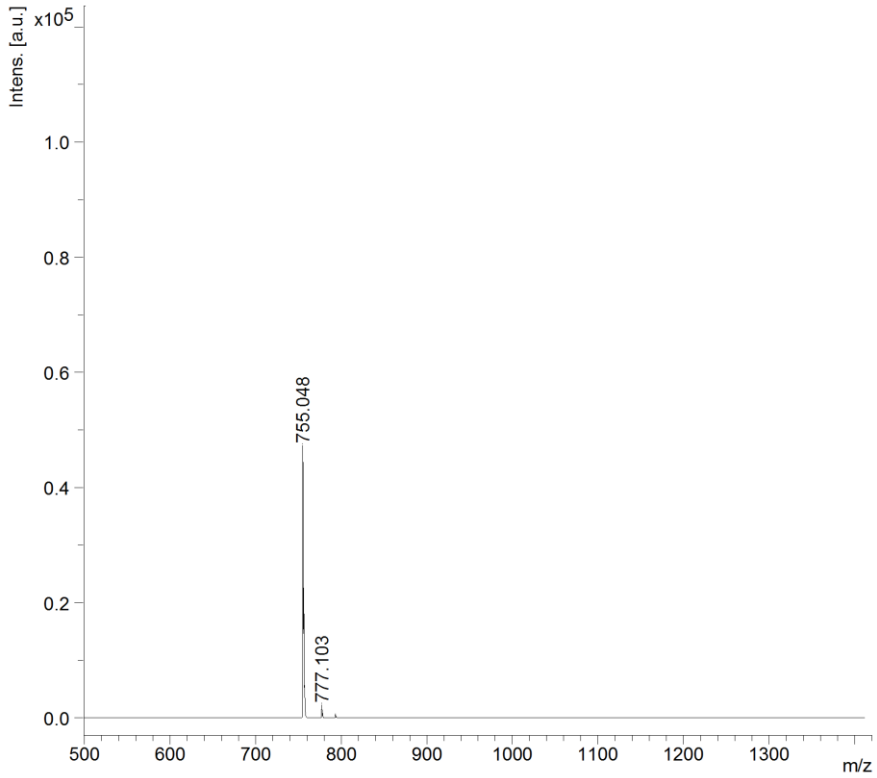

Figure S7. MALDI-TOF mass spectra of **P8**. Calc. $(M+H)^+$ = 754.9793 Da; Obs. $(M+H)^+$ = 755.048 Da.

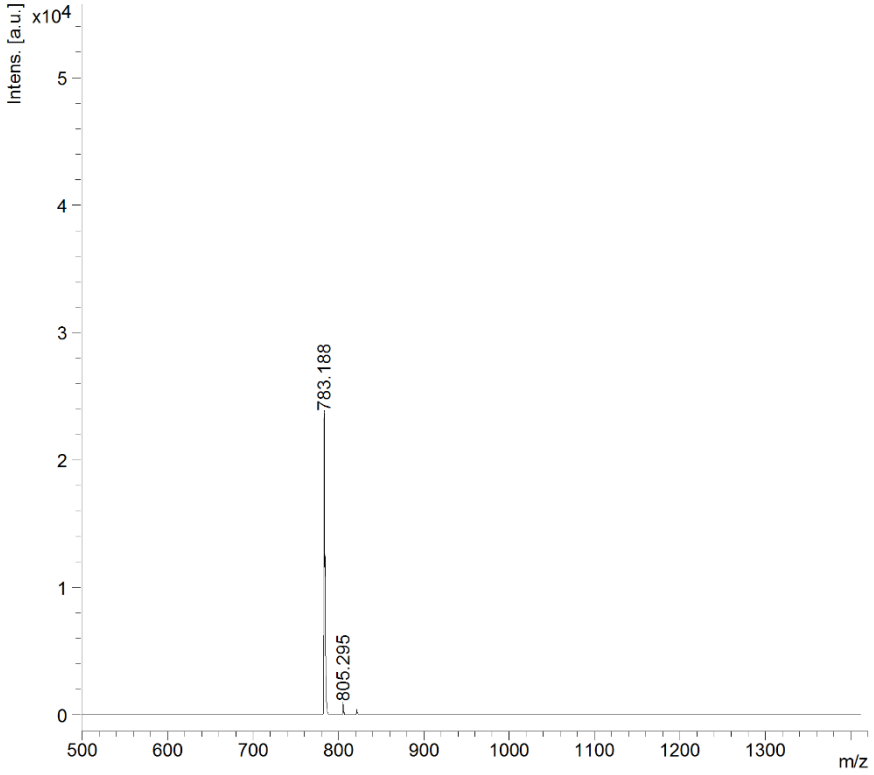

Figure S8. MALDI-TOF mass spectra of **P10**. Calc. $(M+H)^+$ = 782.5650 Da; Obs. $(M+H)^+$ = 783.188 Da, $(M+Na)^+$ =805.295 Da.

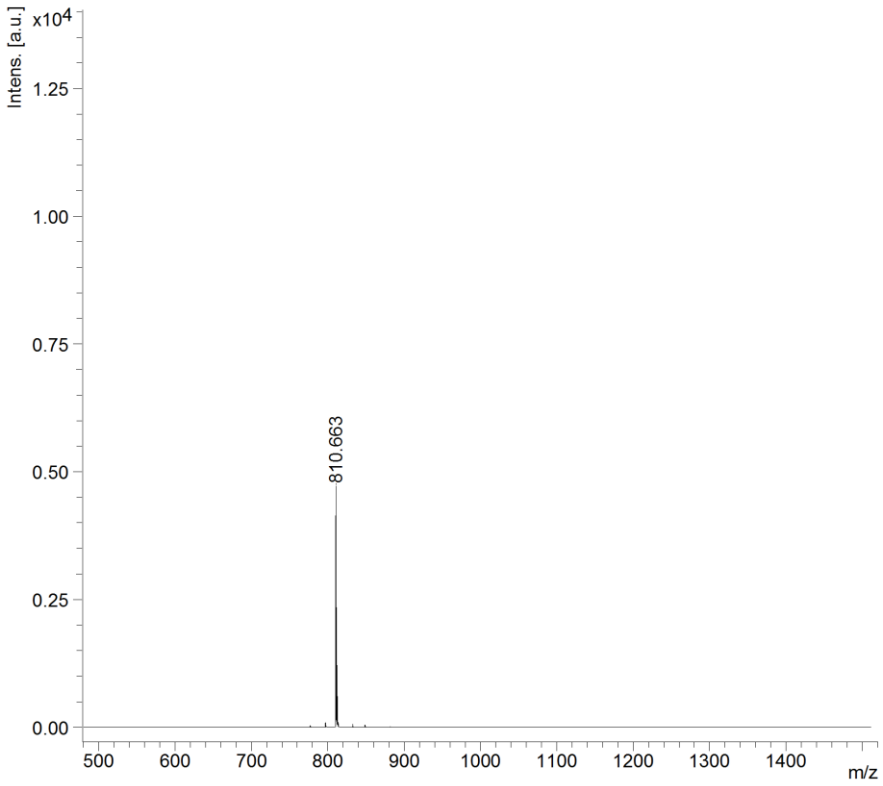

Figure S9. MALDI-TOF mass spectra of **P12**. Calc. $(M+H)^+$= 810.5963 Da; Obs. $(M+H)^+$ = 810.663 Da.

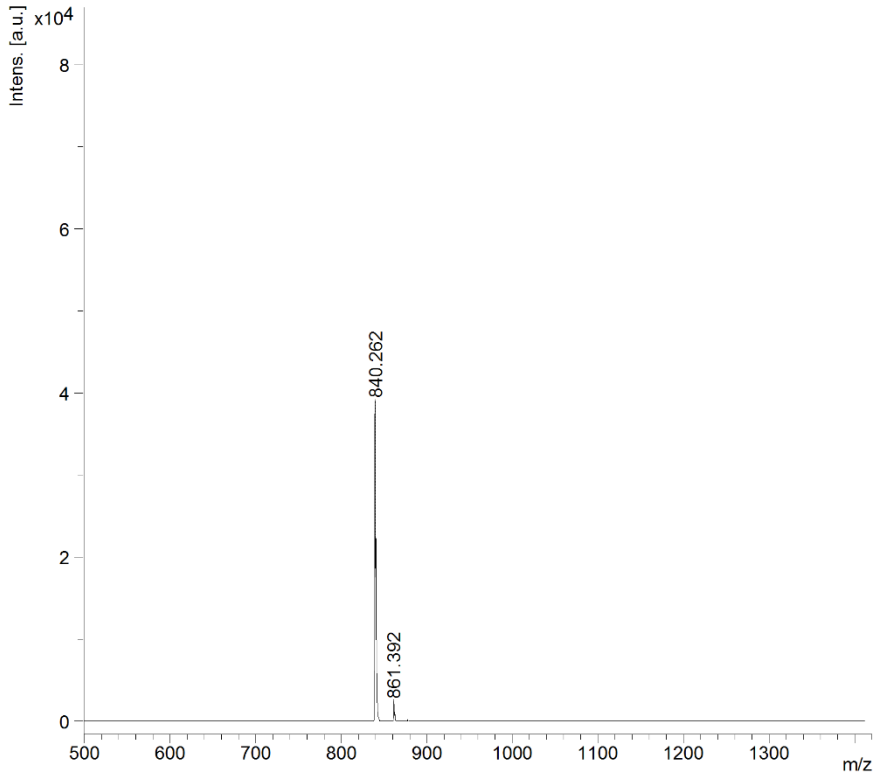

Figure S10. MALDI-TOF mass spectra of **P14**. Calc. $(M+H)^+$ = 838.6276 Da; Obs. $(M+2H)^+$ = 840.262 Da, $(M+Na)^+$ =861.392 Da.

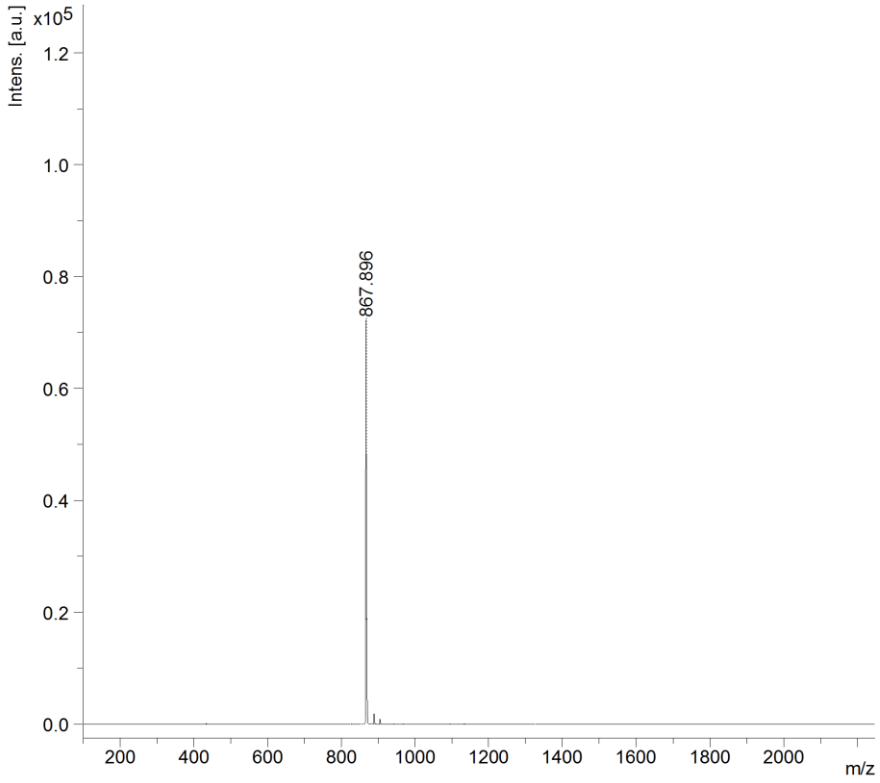

Figure S11. MALDI-TOF mass spectra of **P16**. Calc. $(M+H)^+$ = 867.6589 Da; Obs. $(M+H)^+$ = 867.896 Da.

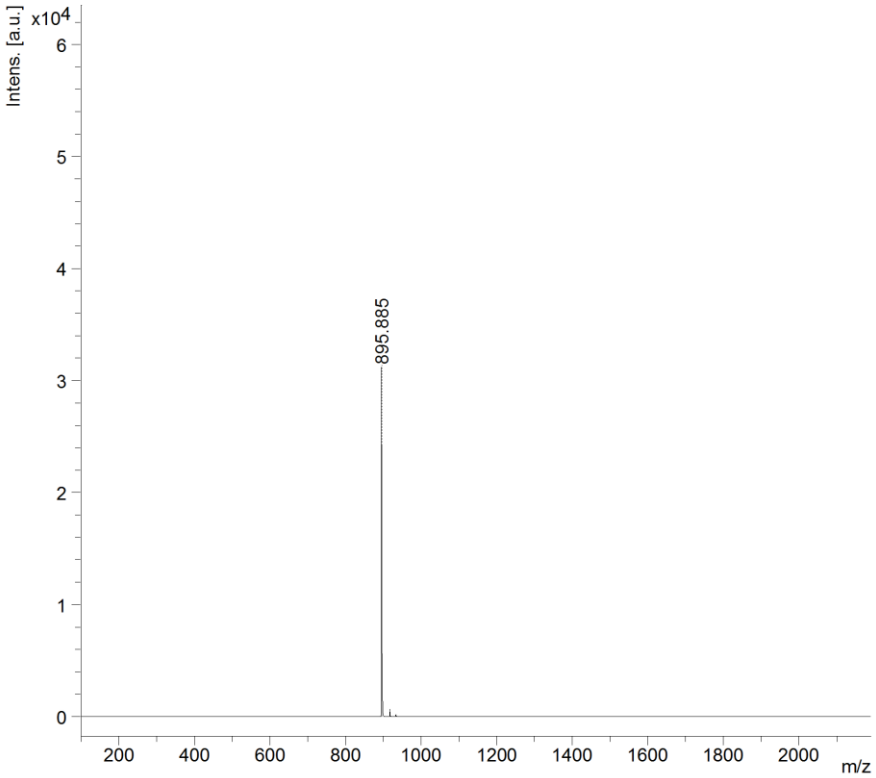

Figure S12. MALDI-TOF mass spectra of **P18**. Calc. $(M+H)^+$ = 894.6902 Da; Obs. $(M+2H)^+$ = 895.885 Da.

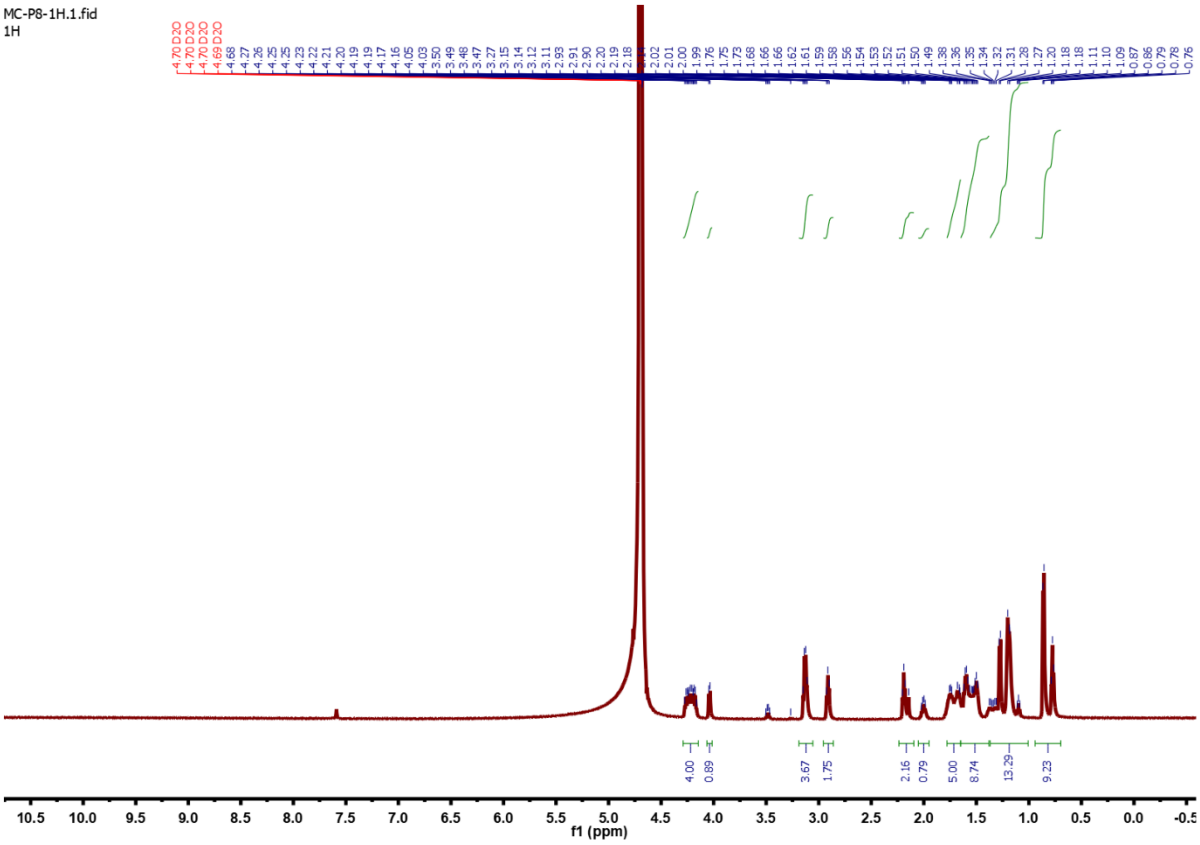

Figure S13. ^{1}H NMR of **P8** in D_2O at room temperature. ^{1}H NMR (600 MHz, D_2O). 0.79-0.90 (9Hs: 3Hs from long-chain -CH_3 and 6Hs from two -CH_3 of Val), 1.2-1.4 (13 Hs: 10Hs from long-chain, 3Hs from -CH_3 of Ala), 1.6-1.8 (14 Hs: 8Hs from Arg, 4Hs from Lys, 2H from long-chain), 2.05-2.2 (3Hs: 1Hs from Val, 2Hs from K), 2.9-3.1 (6Hs: 4Hs from Arg, 2Hs from Lys), 4.06-4.2 (5Hs, 5 αH).

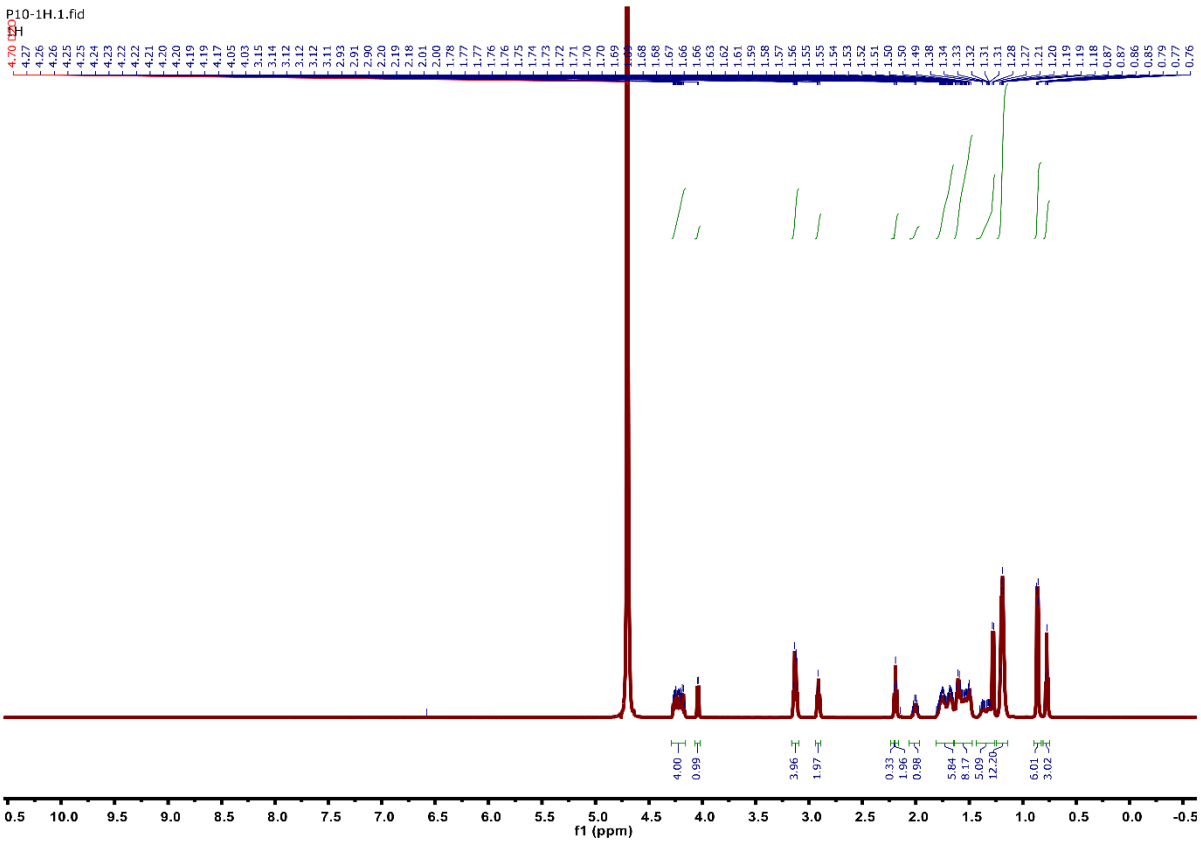

Figure S14. ^{1}H NMR of **P10** in D_2O at room temperature. ^{1}H NMR (600 MHz, D_2O). 0.8-0.89 (9Hs: 3Hs from long-chain -CH_3 and 6Hs from two -CH_3 of Val), 1.2-1.4 (17 Hs: 14Hs from long-chain, 3Hs from -CH_3 of Ala), 1.6-1.8 (14 Hs: 8Hs from Arg, 4Hs from Lys, 2H from long-chain), 2.06-2.2 (3Hs: 1Hs from Val, 2Hs from K), 2.9-3.1 (6Hs: 4Hs from Arg, 2Hs from Lys), 4.07-4.2 (5Hs, 5 αH).

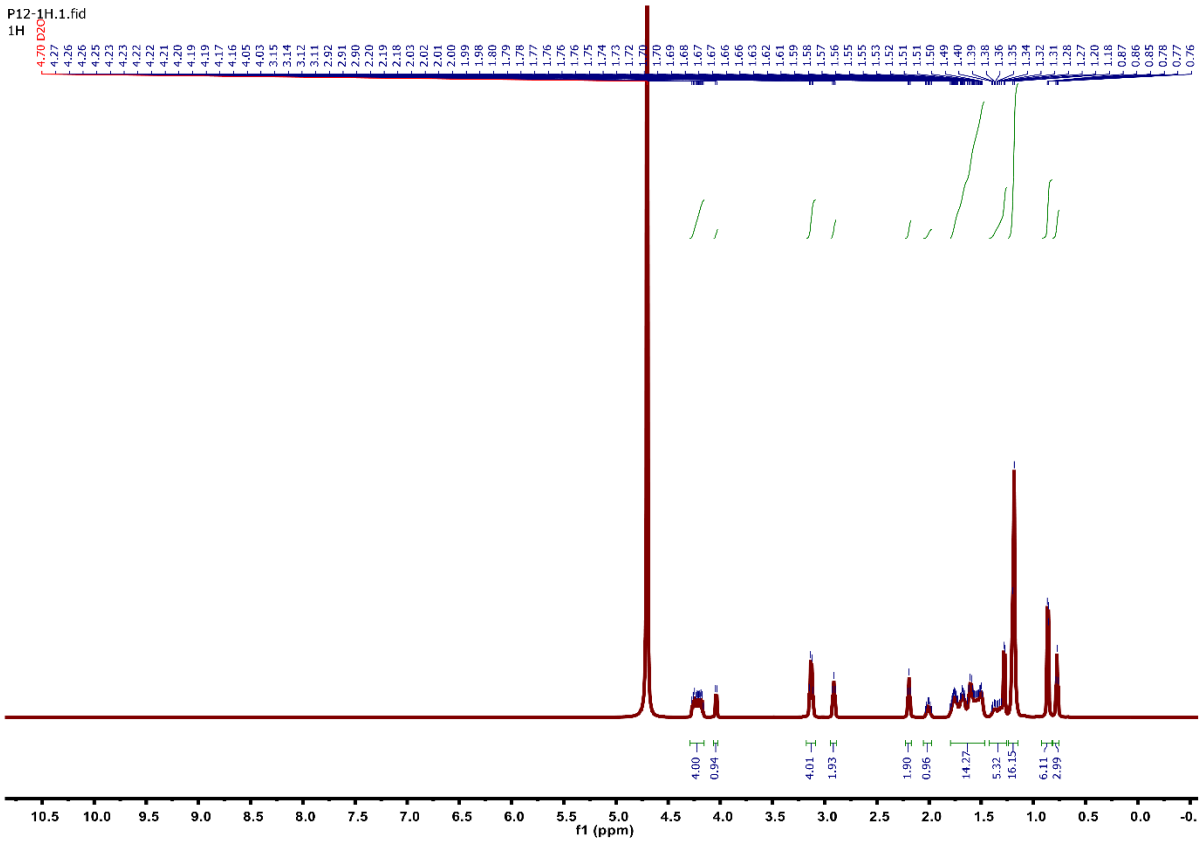

Figure S15. ^{1}H NMR of **P12** in D_2O at room temperature. ^{1}H NMR (600 MHz, D_2O). 0.8-0.92 (9Hs: 3Hs from long-chain -CH_3 and 6Hs from two -CH_3 of Val), 1.2-1.4 (21 Hs: 18Hs from long-chain, 3Hs from -CH_3 of Ala), 1.7 (14 Hs: 8Hs from Arg, 4Hs from Lys, 2H from long-chain), 2.05-2.2 (3Hs: 1Hs from Val, 2Hs from K), 2.9-3.1 (6Hs: 4Hs from Arg, 2Hs from Lys), 4.06-4.2 (5Hs, 5 αH).

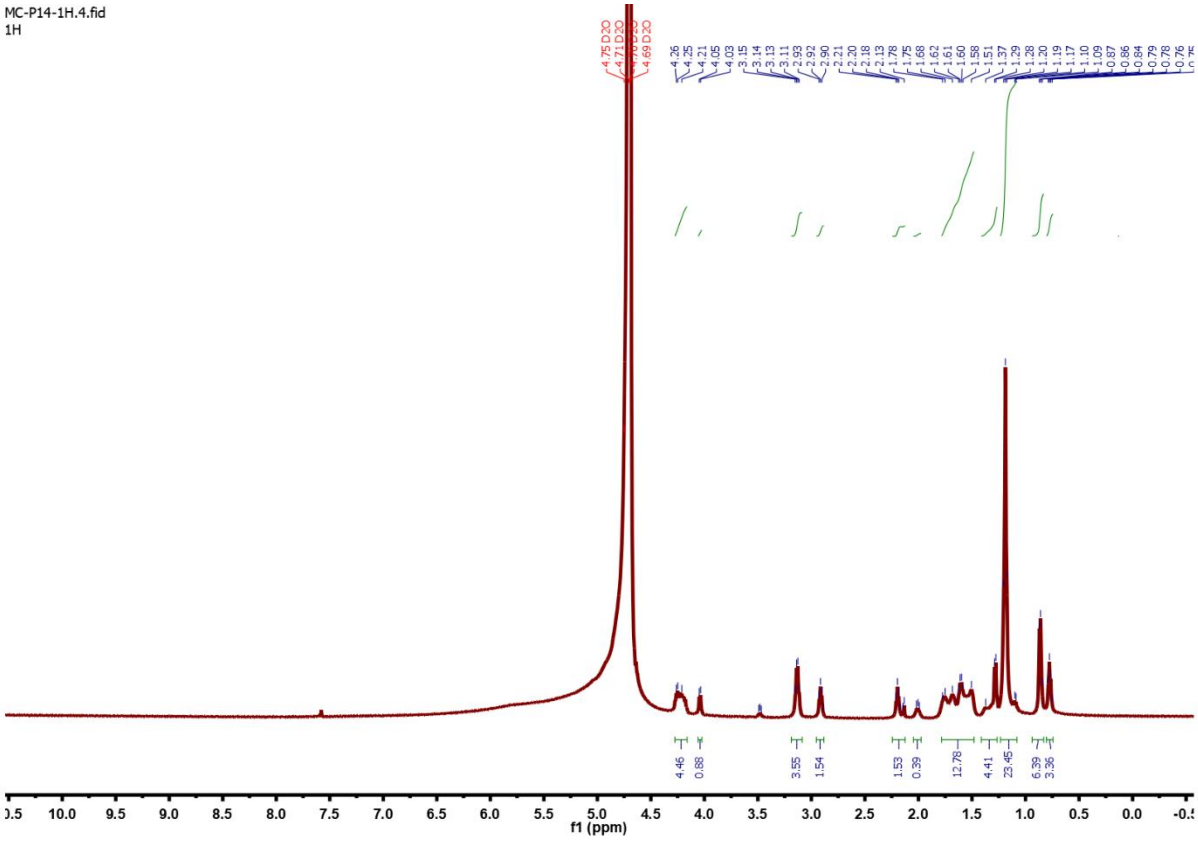

Figure S16. ^{1}H NMR of **P14** in D_2O at room temperature. ^{1}H NMR (600 MHz, D_2O). 0.83-0.93 (9Hs: 3Hs from long-chain -CH_3 and 6Hs from two -CH_3 of Val), 1.2-1.4 (27 Hs: 24Hs from long-chain, 3Hs from -CH_3 of Ala), 1.7 (13 Hs: 8Hs from Arg, 4Hs from Lys, 1H from Val,), 2.05-2.2 (2Hs: 2Hs from K), 2.9-3.1 (6Hs: 4Hs from Arg, 2Hs from Lys), 4.1-4.3 (5Hs, 5 αH).

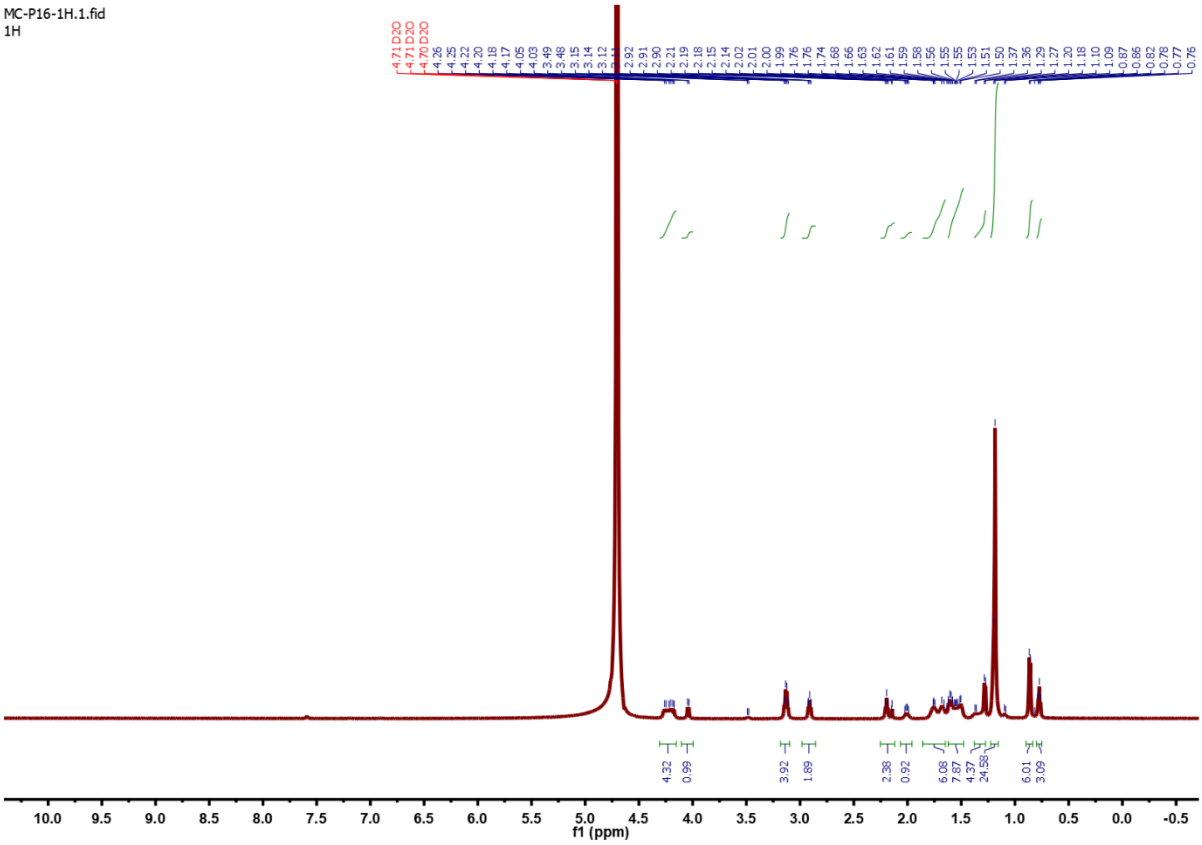

Figure S17. ^{1}H NMR of **P16** in D_2O at room temperature. ^{1}H NMR (600 MHz, D_2O). 0.79-0.89 (9Hs: 3Hs from long-chain -CH_3 and 6Hs from two -CH_3 of Val), 1.22-1.3 (29 Hs: 26Hs from long-chain, 3Hs from -CH_3 of Ala), 1.6-1.8 (14 Hs: 8Hs from Arg, 4Hs from Lys, 2H from long-chain), 2.06-2.25 (3Hs: 1Hs from Val, 2Hs from K), 2.9-3.18 (6Hs: 4Hs from Arg, 2Hs from Lys), 4.1-4.3 (5Hs, 5 αH).

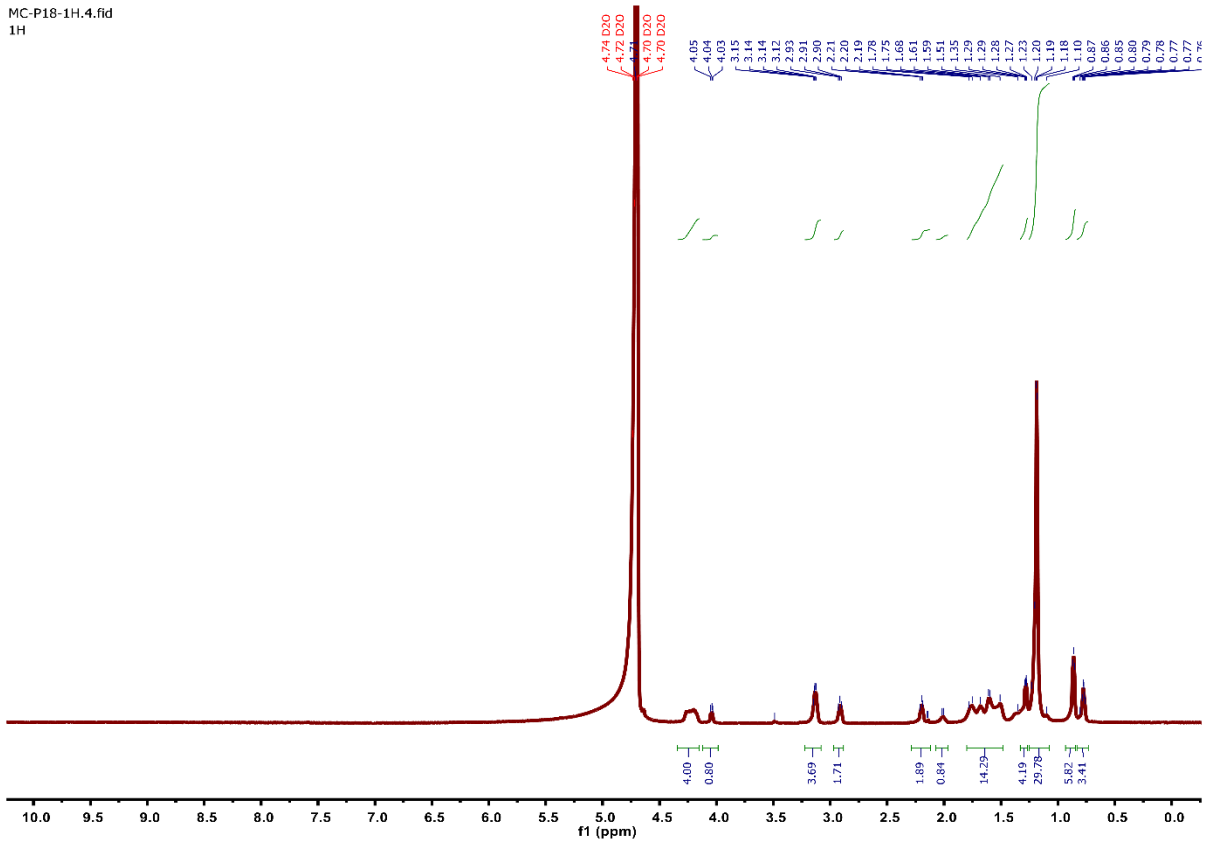

Figure S18. ^{1}H NMR of **P18** in D_2O at room temperature. ^{1}H NMR (600 MHz, D_2O). 0.83-0.93 (9Hs: 3Hs from long-chain -CH_3 and 6Hs from two -CH_3 of Val), 1.25-1.3 (33 Hs: 30Hs from long-chain, 3Hs from -CH_3 of Ala), 1.8 (14 Hs: 8Hs from Arg, 4Hs from Lys, 2H from long-chain), 2.07-2.2 (3Hs: 1Hs from Val, 2Hs from K), 2.9-3.2 (6Hs: 4Hs from Arg, 2Hs from Lys), 4.1-4.3 (5Hs, 5 αH).

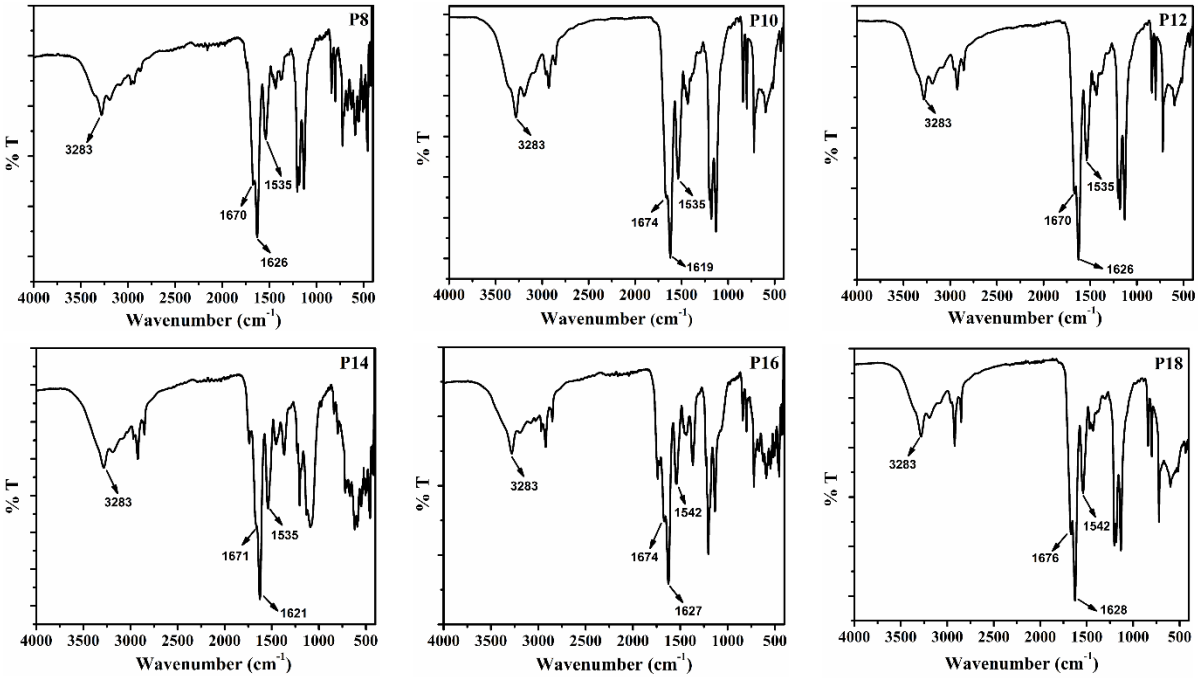

Figure S19. FTIR spectra of **P8-P18.**

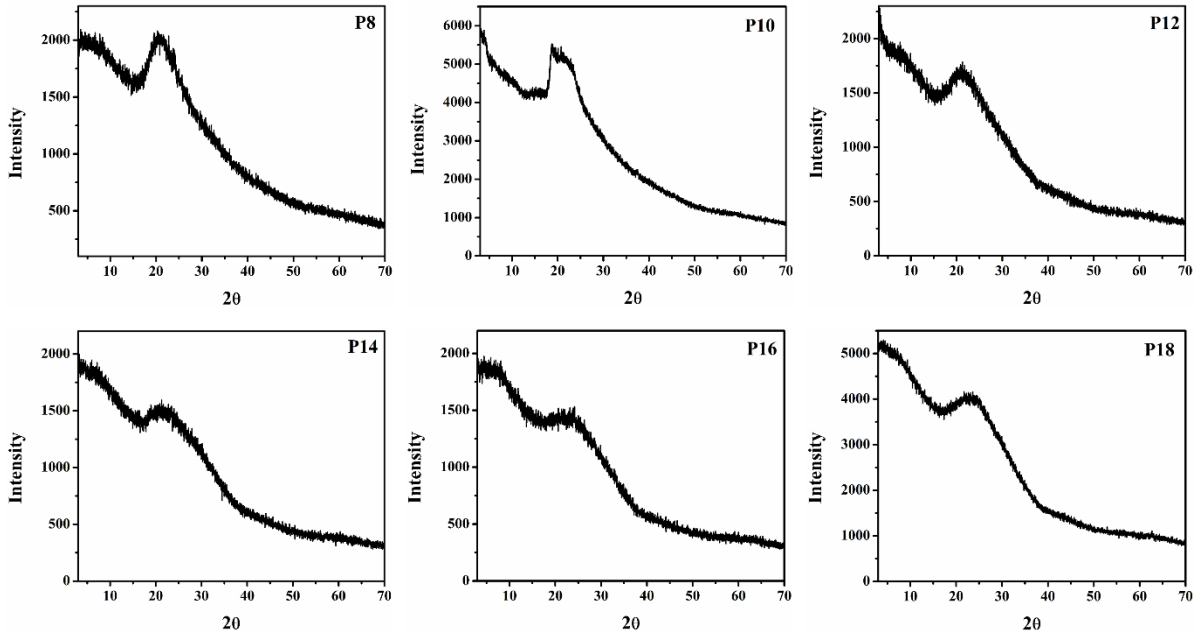

Figure S20. PXRD spectra of **P8-P18.**

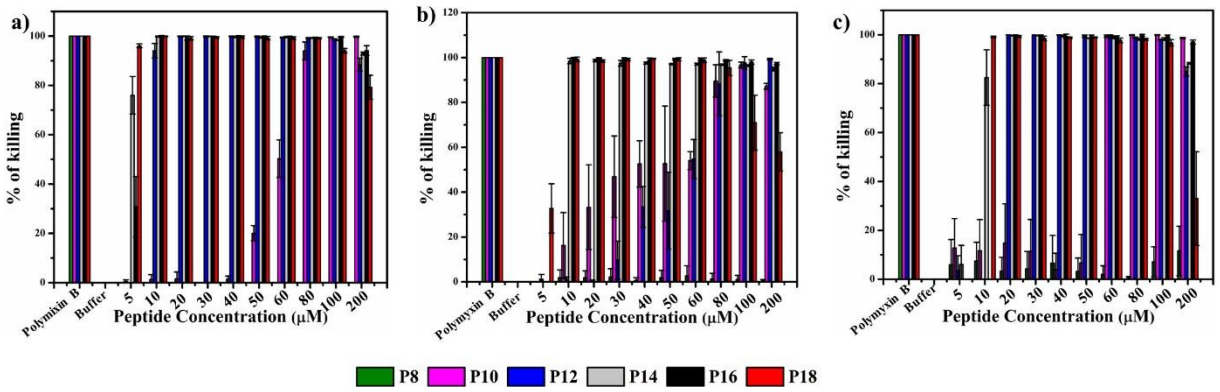

Figure S21. $MIC_{90\%}$ of **P8**-**P18** against a) *P. aeruginosa,* b) *K. pneumonia*, c) *S. aureus* in the absence of salt by Micro broth dilution assay. 10 μM Polymixin B and buffer were taken as positive and negative controls respectively.

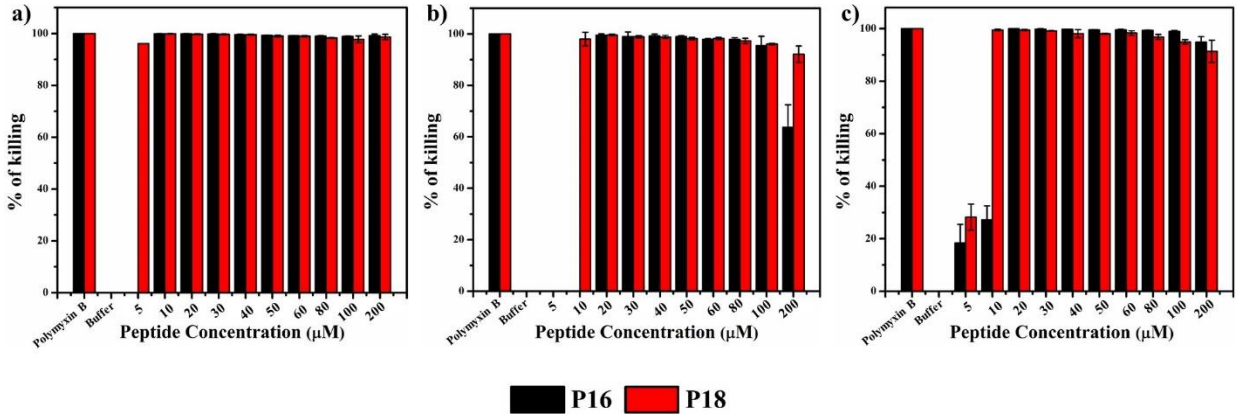

Figure S22. $MIC_{99\%}$ of **P16** and **P18** against a) *P. aeruginosa,* b) *K. pneumonia,* c) *S. aureus* in the presence of salt by Micro broth dilution assay. 10 μM polymixin B and buffer were taken as positive and negative controls respectively.

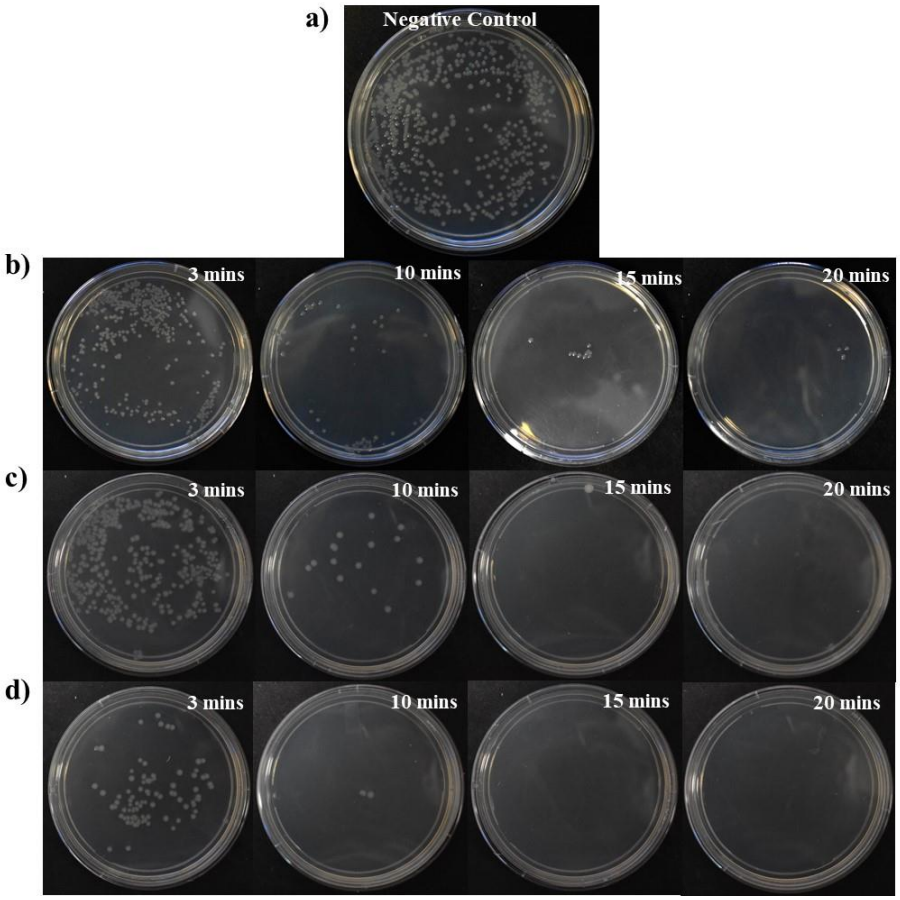

Figure S23. Time kinetics of the bactericidal activity of **P16** and **P18** at their respective MIC99% against *P. aeruginosa* cells. *P. aeruginosa* cells were treated with **P16** and **P18** for different time intervals and cells were spread on NA plate for CFU count after overnight incubation at 37 °C. Bacterial killing percentage was calculated from CFU count of the plate in comparison to the control plate. (a) Negative control (no peptides added) (b) Positive control plate (10 μM Polymyxin B treated cells), (c) plates treated with **P16** at time points 3, 10, 15 and 20 min, respectively and (d) plates treated with **P18** at time points 3, 10, 15 and 20 min, respectively.

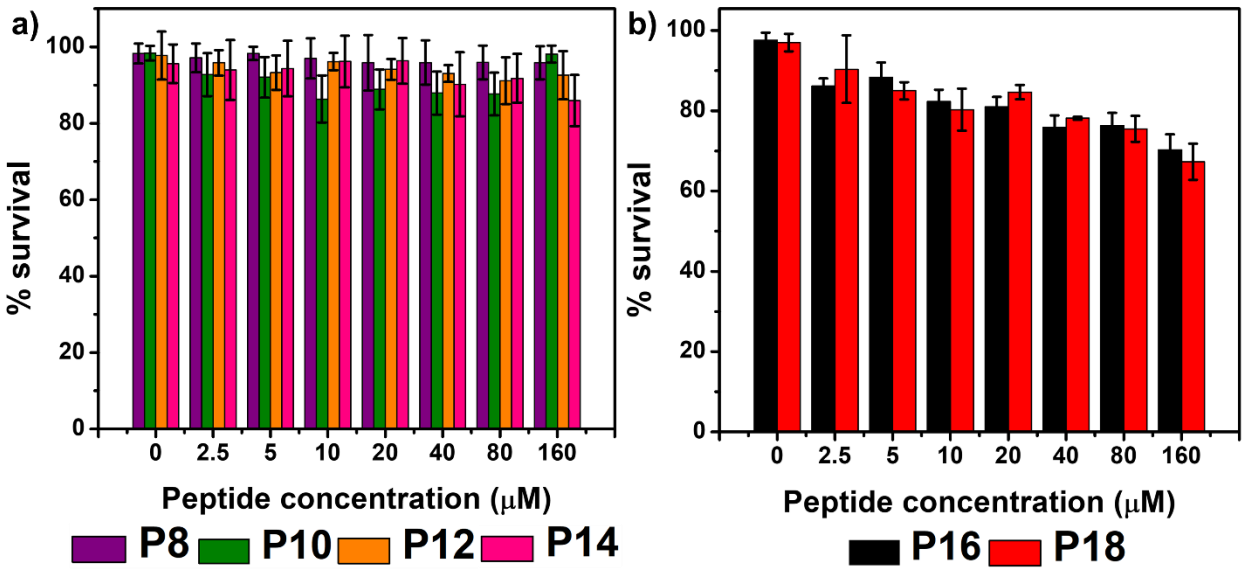

Figure S24. MTT assay of a) **P8**, **P10**, **P12** and **P14** on HEK293 cell line and b) **P16** and **P18** on HeLa cell line. Cells were treated with increasing concentrations of peptides and their viability was measured by monitoring the absorbance at 570 nm. All the experiments were performed in triplicates.

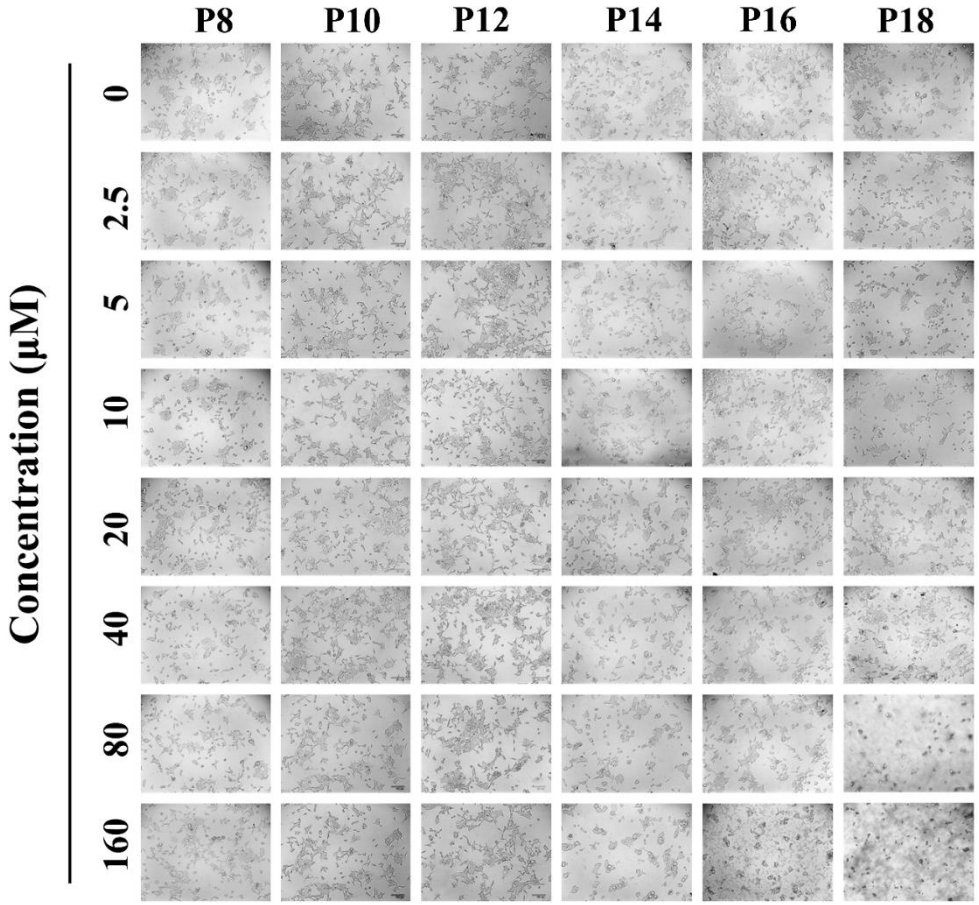

Figure S25. Microscopy images of HEK 293 cells treated with incremental concentrations (0-160 μM) of different lipopeptides **P8-P18** (Scale bar: 100 μm).

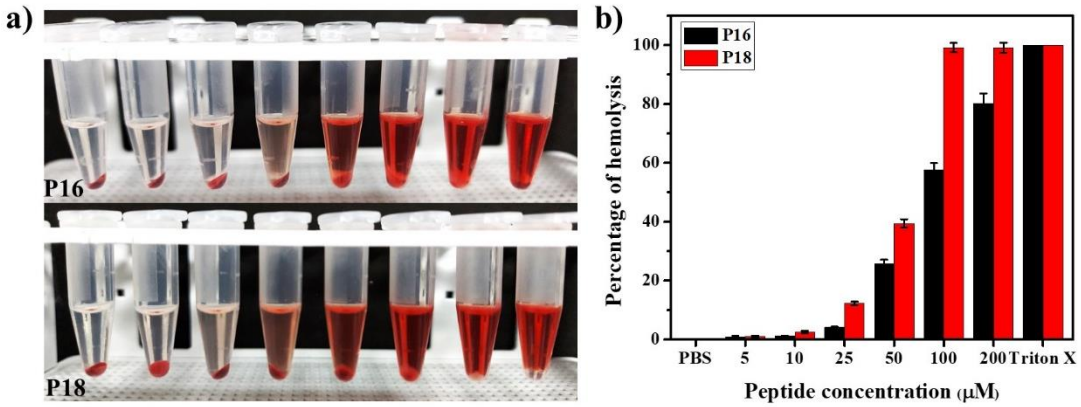

Figure S26. a) Digital images represent haemolytic assay for **P16** and **P18** against human RBC at different peptide concentrations (5, 10, 25, 50, 100, 200 μM), b) Bar diagram showing the % haemolysis for **P16** and **P18** against different concentrations of the peptides. Buffer and Triton-X 100 treated as negative and positive control.

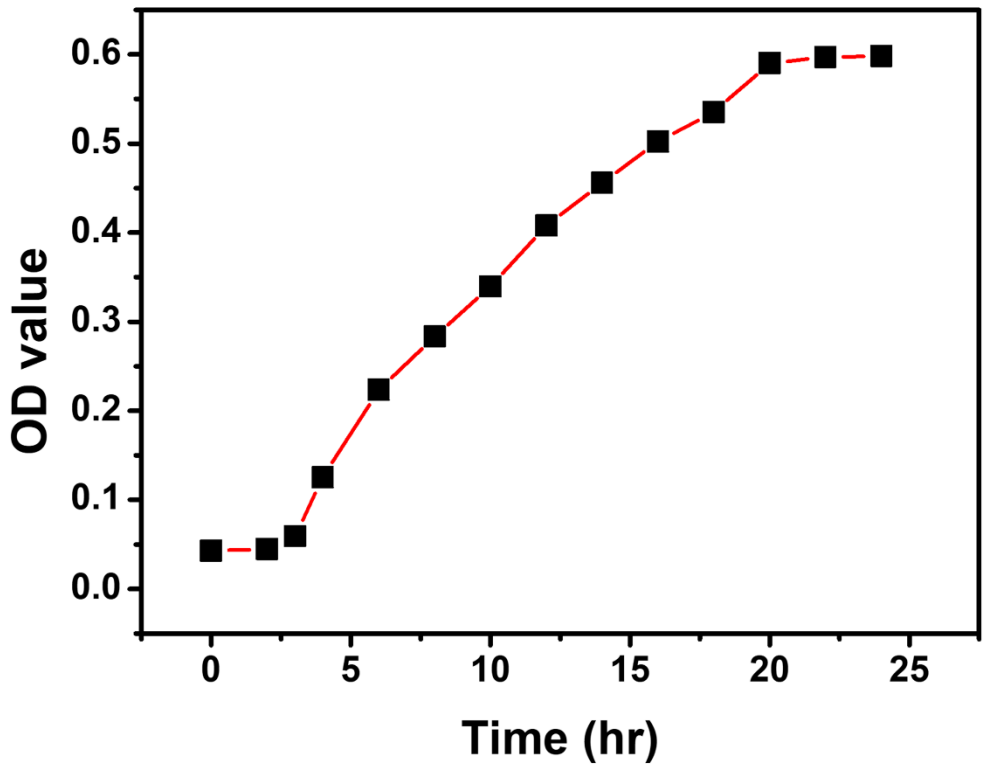

Figure S27. Growth curve of MRSA bacterial strain

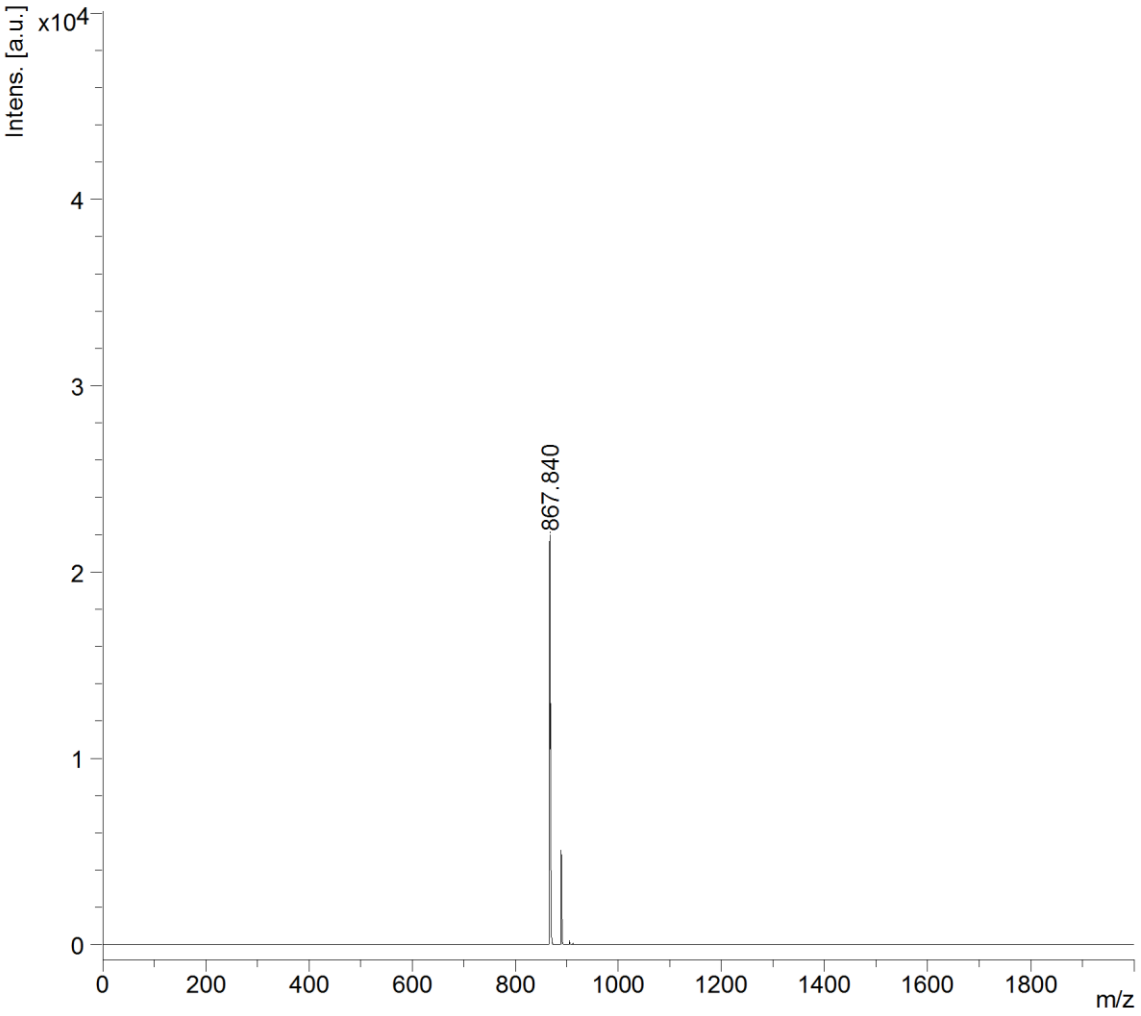

Figure S28a. Control MALDI-TOF mass spectra of **P16** for enzymatic action study. Calc. $(M+H)^+$ = 867.6589 Da; Obs. $(M+H)^+$ = 867.840 Da.

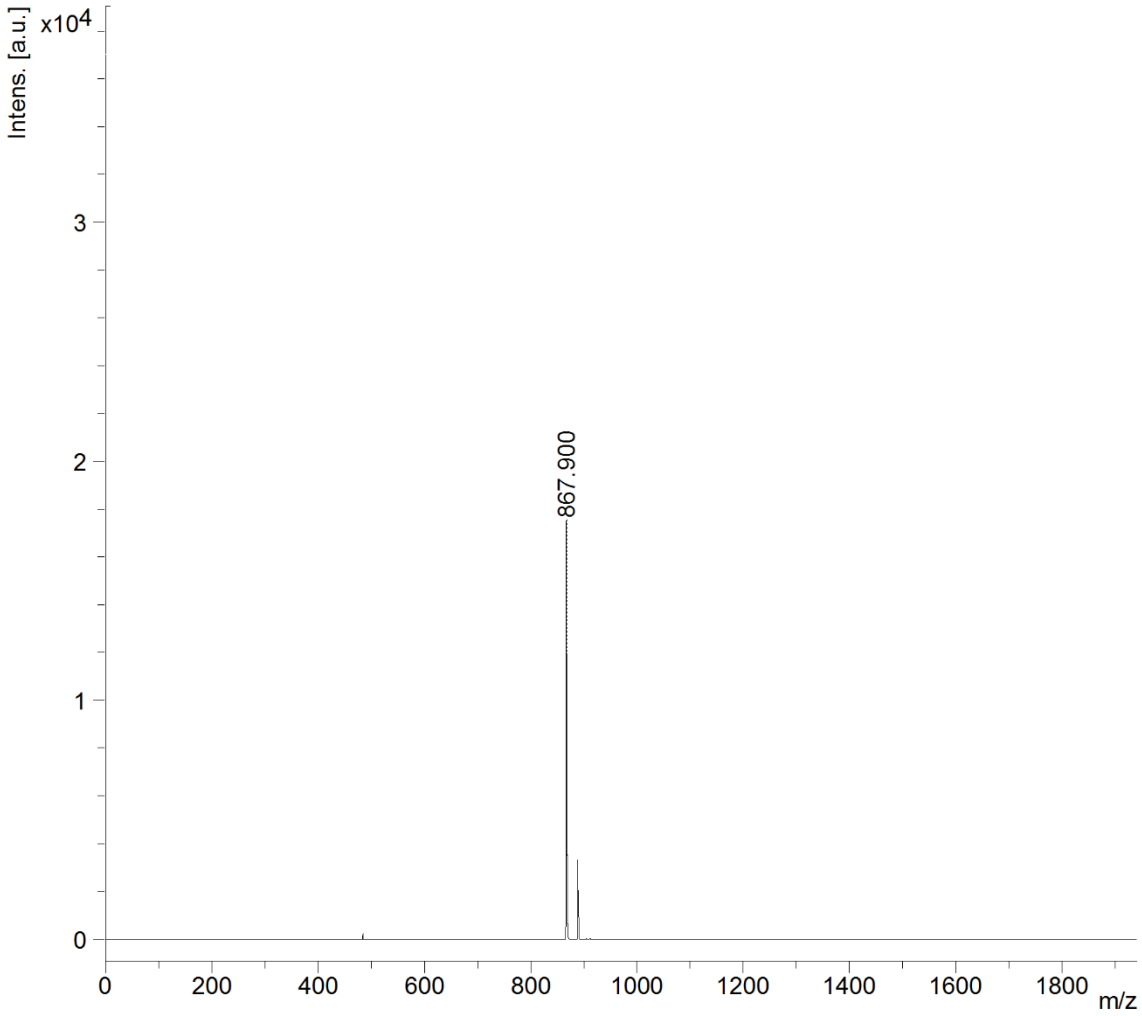

Figure S28b. MALDI-TOF mass spectra of **P16** in presence of proteinase K at 15 mins. Calc. $(M+H)^+$ = 867.6589 Da; Obs. $(M+H)^+$ = 867.900 Da.

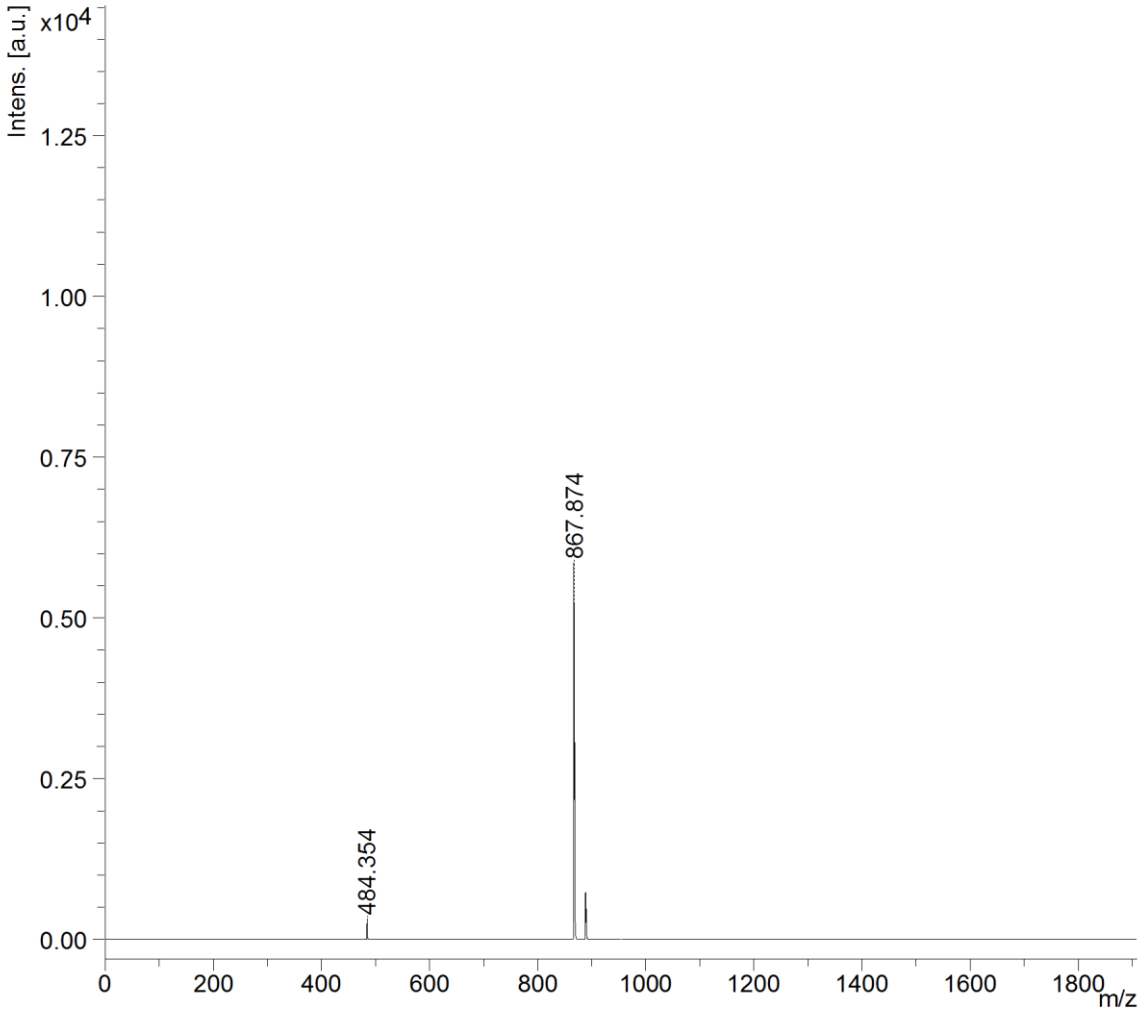

Figure S28c. MALDI-TOF mass spectra of **P16** in presence of proteinase K at 1 hr. Calc. $(M+H)^+$ = 867.6589 Da; Obs. $(M+H)^+$ = 867.874 Da. m/z 484.357 corresponds to $(M+2H^+)$ of the fragment C_{16}-AR.

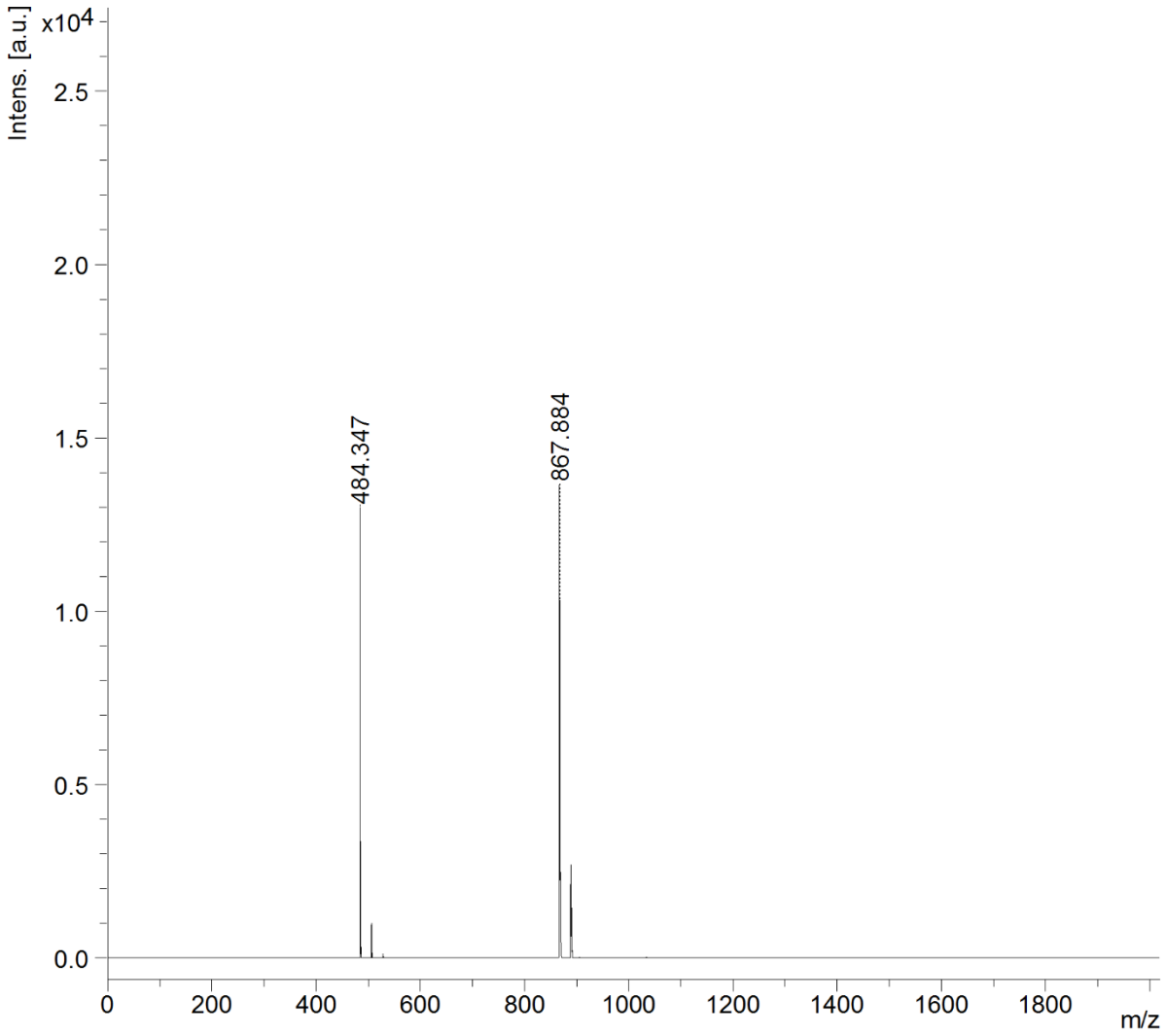

Figure S28d. MALDI-TOF mass spectra of **P16** in presence proteinase K at 3 hr. Calc. $(M+H)^+$ = 867.6589 Da; Obs. $(M+H)^+$ = 867.884 Da. m/z 484.347 corresponds to $(M+2H^+)$ of the fragment C_{16}-AR.

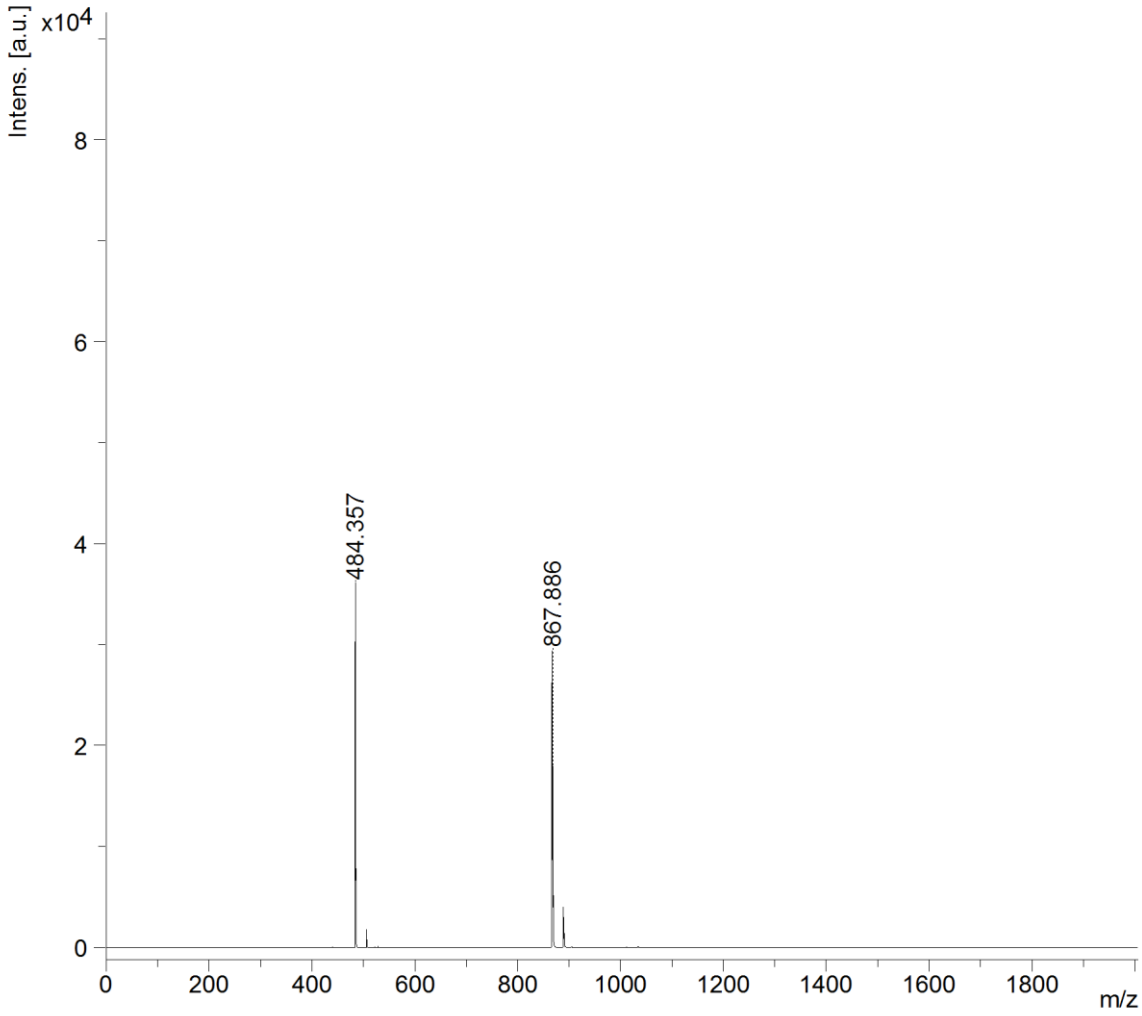

Figure S28e. MALDI-TOF mass spectra of **P16** in presence of proteinase K at 6 hr. Calc. $(M+H)^+$ = 867.6589 Da; Obs. $(M+H)^+$ = 867.884 Da. m/z = 484.357 corresponds to $(M+2H^+)$ of the fragment C_{16}-AR.

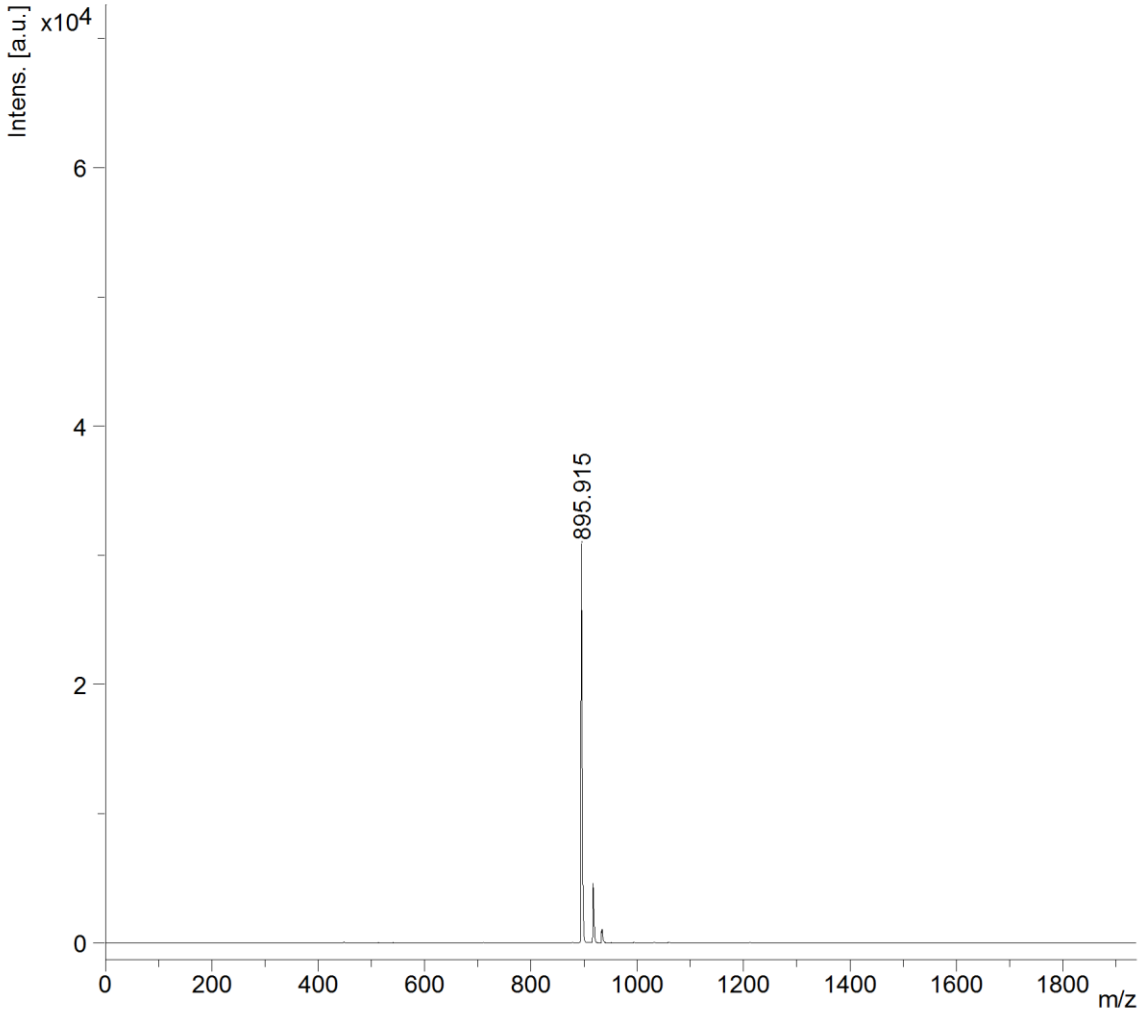

Figure S29a. Control MALDI-TOF mass spectra of **P18** for enzymatic action study. Calc. $(M+2H)^+$ = 895.6902Da; Obs. $(M+2H)^+$ = 895.915 Da.

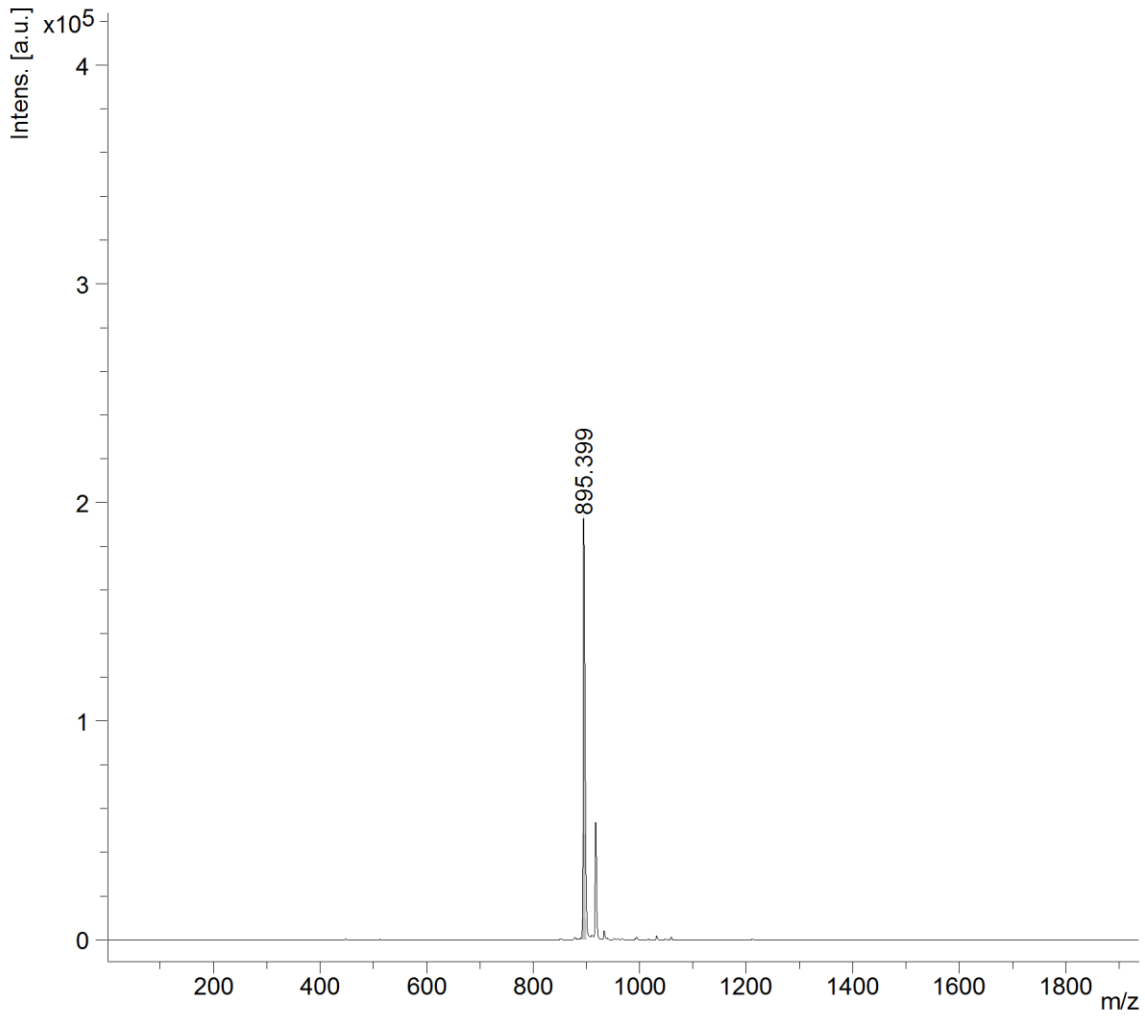

Figure S29b. MALDI-TOF mass spectra of **P18** in presence of proteinase K at 15 min. Calc. $(M+2H)^+$ = 895.6902 Da; Obs. $(M+2H)^+$ = 895.399 Da.

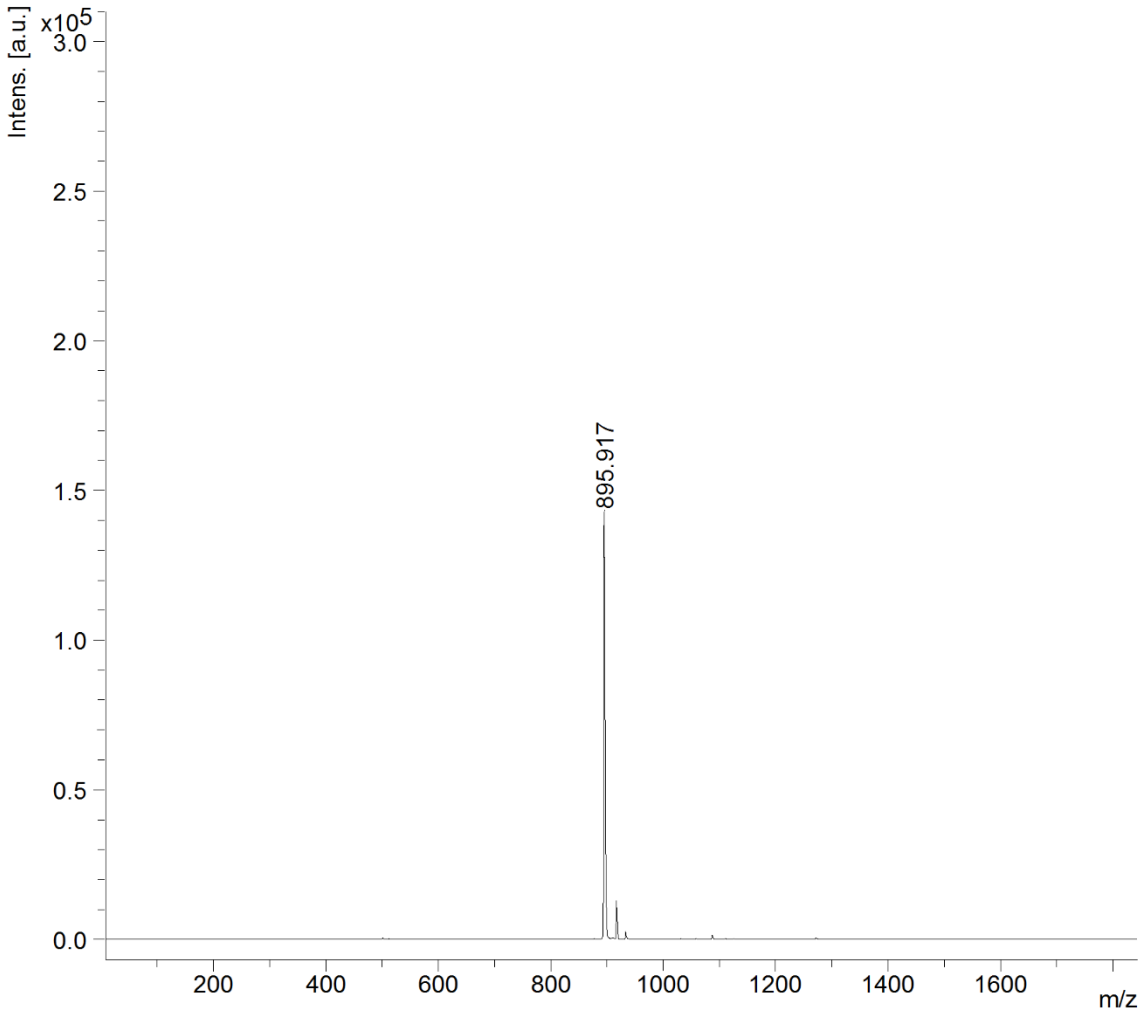

Figure S29c. MALDI-TOF mass spectra of **P18** in presence of proteinase K at 1 hr. Calc. $(M+2H)^+$ = 895.6902 Da; Obs. $(M+2H)^+$ = 895.917 Da.

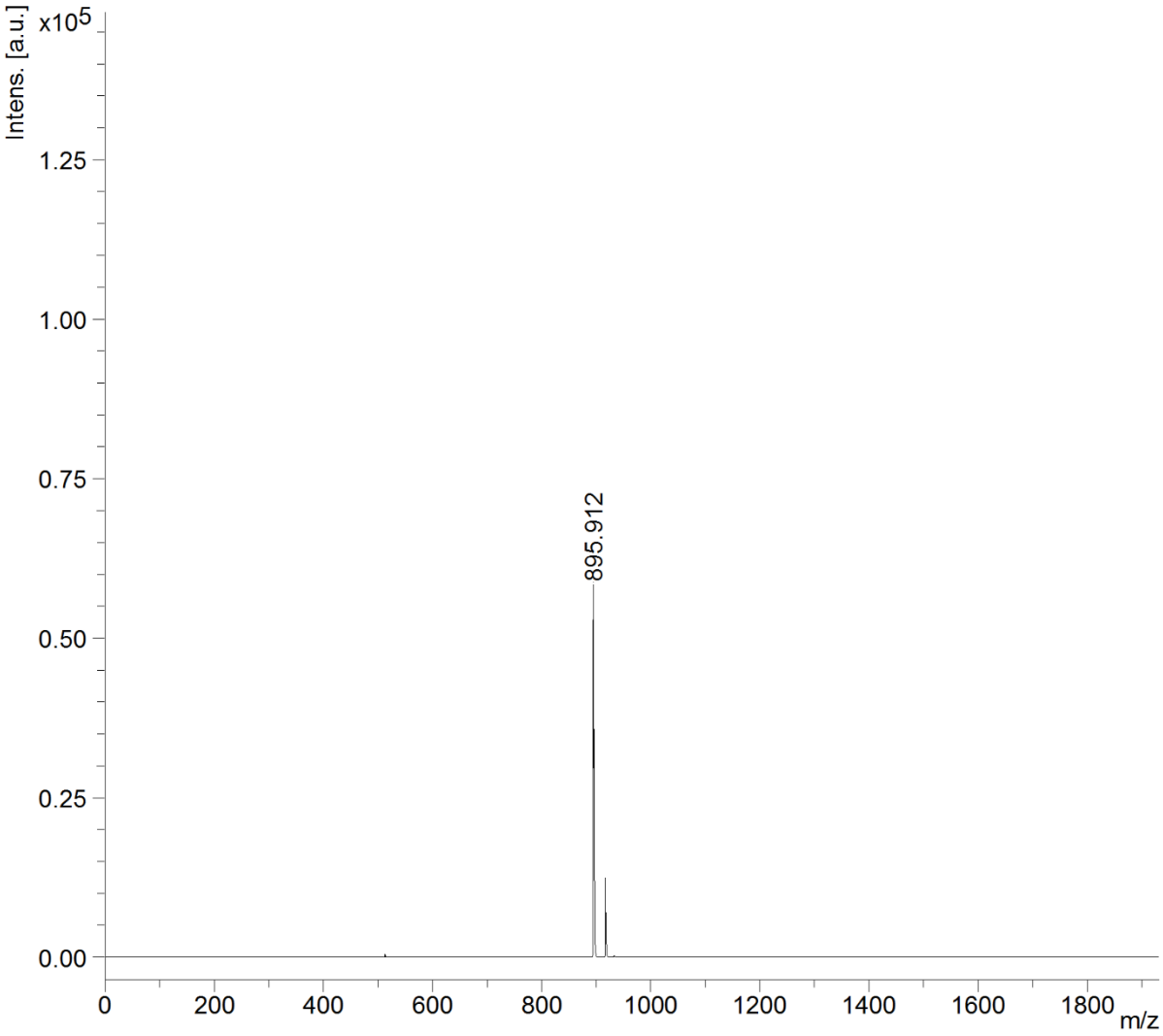

Figure S29d. MALDI-TOF mass spectra of **P18** in presence of proteinase K at 3 hr. Calc. $(M+2H)^+$ = 895.6902 Da; Obs. $(M+2H)^+$ = 895.912 Da.

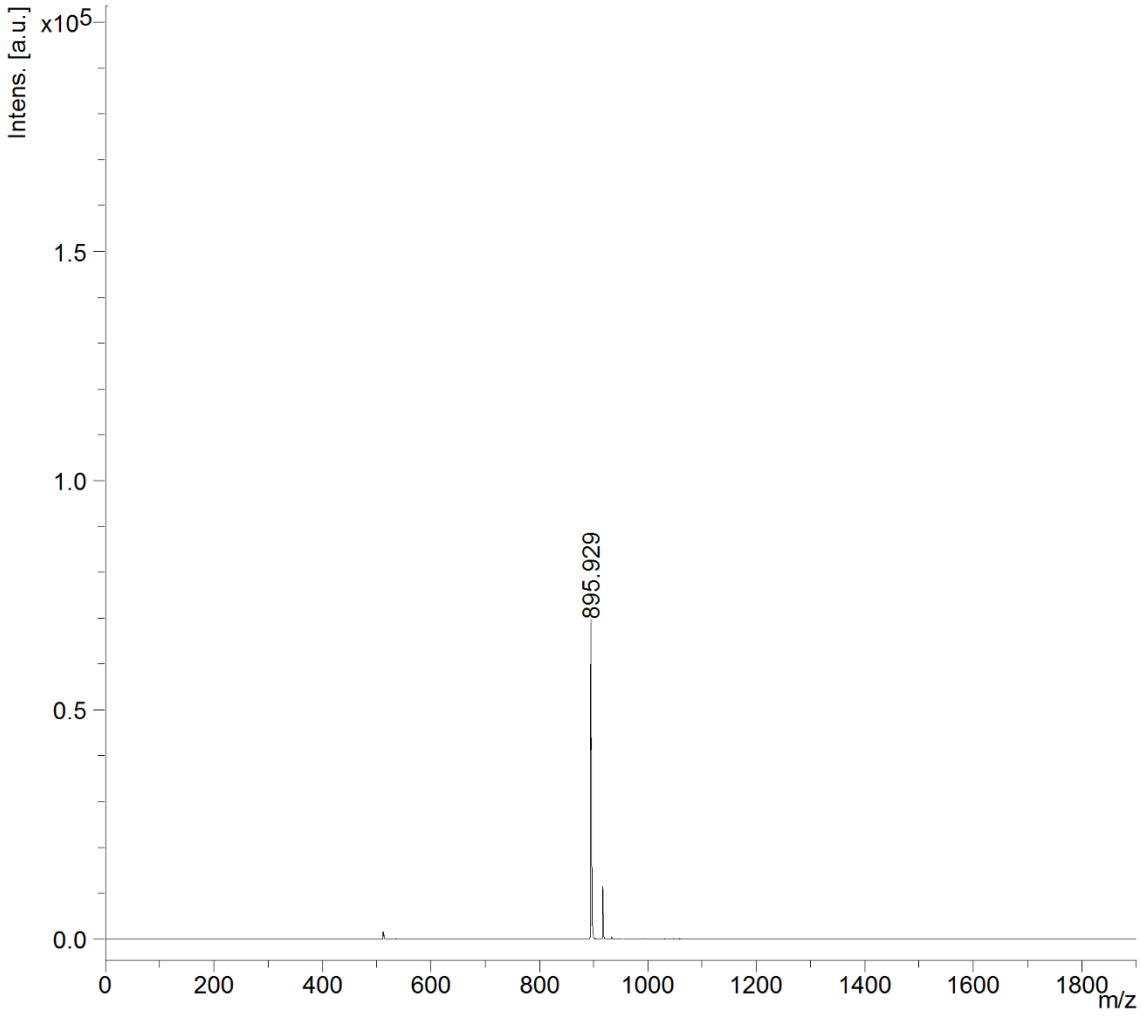

Figure S29e. MALDI-TOF mass spectra of **P18** in presence of proteinase K at 6 hr. Calc. $(M+2H)^+$ = 895.6902 Da; Obs. $(M+2H)^+$ = 895.929 Da.

Table S1. 2θ and d-spacing values from PXRD

Peptides	**P8**	**P10**	**P12**	**P14**	**P16**	**P18**
2θ	20.75	20.88	20.96	21.78	23.028	23.7
d spacing (Å)	4.27	4.24	4.241	4.07	3.85	3.8